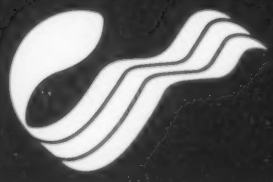


Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Canada

Scientific Excellence • Resource Protection & Conservation • Benefits for Canadians

Excellence scientifique • Protection et conservation des ressources • Bénéfices aux Canadiens

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial Policy

The *Journal* publishes original research articles, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

A charge is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Guides

A guide for authors and typists appears in the first issue of each volume and is available free from the *Journal*.

Subscriptions

The *Journal* is issued monthly to form annual volumes of twelve numbers plus Supplements. Orders and inquiries regarding subscriptions must be sent directly to Canada Communication Group — Publishing, Supply and Services Canada, Ottawa, Ontario, Canada K1A 0S9. Prepayment is required to the order of "The Receiver General for Canada."

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Printed in Canada by: The Runge Press Limited, Ottawa

Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

Politique de rédaction

Le *Journal* publie des articles fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, b) des arbitres possibles et c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Les manuscrits acceptés sont sujets à des frais de publication. Quand un auteur présente un texte, il doit préciser que ces frais seront acceptés. On trouvera dans une récente livraison du *Journal* les taux par page en vigueur.

Guides

Le guide des auteurs et des secrétaires d'auteurs paraît dans la première livraison de chaque volume et est offert gratuitement par le *Journal*.

Abonnement

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros, et offre en plus jusqu'à deux suppléments par année. On doit faire parvenir les commandes et demandes de renseignements concernant les abonnements au Groupe Communication Canada — Édition, pprovisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9. Les paiements doivent être faits à l'avance à l'ordre du Receveur général du Canada.

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 48, No. 2, February 1991 | **Volume 48, nº 2, février 1991**

Scientific Publications/Publications scientifiques

D. G. Cook, Ph.D. — *Editor/Rédacteur*
J. Camp/G. J. Neville/P. M. Burke/M. Milne — *Editorial and Publishing Services/Services de rédaction et d'édition*
P. E. K. Symons, Ph.D. — *Consulting Editor/Rédacteur-conseil (Victoria, B.C.)*

Associate Editors/Rédacteurs associés

J. R. M. Kelso, Ph.D. — *Department of Fisheries and Oceans, Sault Ste. Marie*
C. D. Levings, Ph.D. — *Department of Fisheries and Oceans, West Vancouver*
J. D. Neilson, Ph.D. — *Department of Fisheries and Oceans, St. Andrews*
P. Schwinghamer, Ph.D. — *Department of Fisheries and Oceans, St. John's*
N. H. F. Watson, Ph.D. — *Department of Fisheries and Oceans, Halifax*
D. J. Noakes, Ph.D. — *Department of Fisheries and Oceans, Nanaimo*

Editorial Board/Conseil de rédaction

S. J. Smith — *Department of Fisheries and Oceans, Dartmouth* (1988–1991)
J. C. Roff — *University of Guelph* (1990–1992)
L. M. Dickie — *Department of Fisheries and Oceans, Dartmouth* (1990–1992)

Editorial Office:

Department of Fisheries and Oceans
Communications Directorate
Scientific Publications
200 Kent Street, 14th Floor
Ottawa, Canada K1A 0E6
Telephone (613) 993–2209
FAX (613) 990-1866

Bureau de rédaction :

Ministère des Pêches et des Océans
Direction générale des communications
Publications scientifiques
200, rue Kent, 14e étage
Ottawa, Canada K1A 0E6
Numéro de téléphone (613) 993–2209
FAX (613) 990-1866

The *Journal* is abstracted or indexed in:/Le *Journal* est résumé ou signalé dans :

Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Oceanic Abstracts, and Science Citation Index

Effects of Increases in Freshwater Temperature on Loss of Smolt Characteristics in Atlantic Salmon (*Salmo salar*)

J. Duston, R. L. Saunders, and D. E. Knox[1]

Department of Fisheries and Oceans, Biological Sciences Branch, Biological Station, St. Andrews, N.B. E0G 2X0, Canada

Duston, J., R. L. Saunders, and D. E. Knox. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 48: 164–169.

Two-year-old (2⁺) Atlantic salmon (*Salmo salar*) raised under ambient temperature and simulated natural photoperiod (45°N) completed smolting between February and May, exhibiting significant increases in gill Na^+, K^+-ATPase activity and salinity tolerance (96 h, 37.5 ppt) but no significant changes in plasma osmolality. On May 2 the smolts were divided into four groups (each $n = 80$) and subjected to a rapid (2–3 h) increase in temperature from ambient (5°C) to 10, 13, or 16°C or remained ambient (control) which rose to 12°C by the end of the experiment on June 5. In the 10, 13, and 16°C groups, mean gill Na^+, K^+-ATPase activity (micromoles inorganic phosphorus per milligram protein per hour) declined significantly from 6.6 units on May 2 to 4.3, 3.6, and 2.3 units, respectively, on May 23. In the control group, Na^+, K^+-ATPase activity showed no significant changes during the study. Plasma osmolality was maintained in all groups between 290 and 304 mosmol·kg^{-1}. Salinity tolerance tests revealed a significant decline in survival in the 16°C group from May 2 and in the 10°C, 13°C, and control groups from May 13, but no significant differences were observed among the three groups. The results support the hypothesis that increases in freshwater temperature accelerate the loss of hypoosmoregulatory capacity.

La smoltification de saumons de l'Atlantique (*Salmo salar*) de 2 ans (2⁺) élevés dans des conditions de température ambiante et de photopériode naturelle simulée (45°N) a eu lieu entre février et mai. Les poissons ont montré une nette augmentation de l'activité de la Na^+, K^+-ATPase activity des ouïes et de la tolérance à la salinité (96 h, 37,5 ppt), mais aucune augmentation nette de l'osmolalité du plasma. Le 2 mai, on a divisé les saumoneaux en quatre groupes de 80 individus et on les a soumis à une augmentation rapide (2–3 h) de la température, soit d'une température ambiante de 5°C à 10, 13, ou 16°C. On a gardé le groupe-témoin à la température ambiante qui est passée à 12°C à la fin de l'expérience, soit le 5 juin. Dans le cas des groupes exposés à une température de 10, 13 et 16°C, l'activité moyenne de la Na^+, K^+-ATPase activity dans les ouies (micromoles de phosphore inorganique par milligramme de protéine par heure) a nettement baissé : elle est passée de 6,6 unités le 2 mai à 4,3, 3,6 et 2,3 unités, respectivement, le 23 mai. Dans le groupe-témoin, l'activité de la Na^+, K^+-ATPase activity n'a pas nettement varié au cours de l'étude. L'osmolalité du plasma s'est maintenu entre 290 et 304 mosmol·kg^{-1} chez tous les groupes. Les résultats des tests de tolérance à la salinité ont révélé une baisse significative du taux de survie chez le groupe exposé à une température de 16°C à partir du 2 mai ainsi que chez les groupes exposés à des températures de 10 et 13°C et le groupe-témoin à partir du 13 mai, mais aucune différence significative n'a été observée parmi les trois groupes. Les résultats étayent l'hypothèse que l'augmentation de la température des eaux douces accélère la perte de la capacité d'hypoosmorégulation.

Received April 24, 1990
Accepted August 20, 1990
(JA551)

Reçu le 24 avril 1990
Accepté le 20 août 1990

The life cycle of the Atlantic salmon (*Salmo salar*) and other salmonid species is typically divided between freshwater and marine environments. Under natural conditions, spawning occurs in fresh water and the juvenile salmon, known as parr, remain in this habitat for 1 or more yr before undergoing parr–smolt transformation. Smolting which occurs in fresh water and preadapts the fish to the sea is usually completed in the spring and consists of an array of physiological and behavioural changes (Hoar 1976, 1988; Wedemeyer et al. 1980; McCormick and Saunders 1987). Amongst these changes is the development of hypoosmoregulatory mechanisms which include an elevation in gill Na^+, K^+-activated, Mg^{2+}-dependent ATPase (Na^+, K^+-ATPase) activity (Zaugg and McLain 1970; Saunders and Henderson 1978; Primmett et al. 1988), an enzyme found in high concentrations in the chloride cells of euryhaline teleosts adapted to saltwater and believed to be involved in the excretion of excess monovalent ions from the body fluids (Foskett et al. 1983; De Renzis and Bornancin 1984; Karnaky 1986).

The timing of completion of smoltification appears to be controlled by an endogenous circannual rhythm entrainable by the annual photoperiod cycle (see Duston and Saunders 1990). If downstream migrating smolts fail to reach the sea, or are prevented from migrating by retention in fresh water, they lose at least some of their smolt characteristics (Folmar et al. 1982). This apparent abandonment of smolt status which is often associated with elevations in water temperature above 10°C has been quantified in terms of declines in salinity tolerance (Conte and Wagner 1965; Wagner 1974; Harache et al. 1980), salinity preference (Baggerman 1960; McInerney 1964), migratory behaviour (Zaugg and Wagner 1973), alteration in urine pro-

[1]Present address: Connors Brothers Limited, Blacks Harbour, N.B. E0G 1H0, Canada.

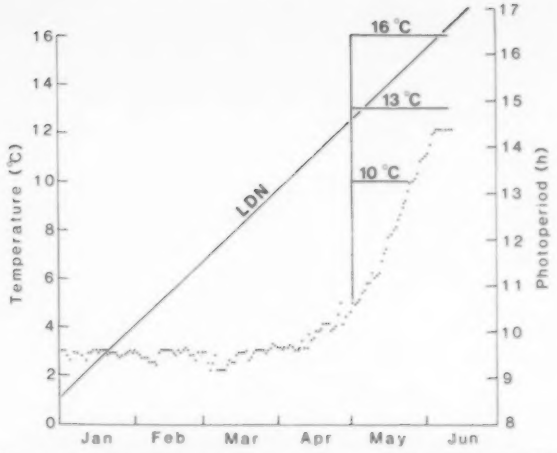


FIG. 1. Experimental temperature regimes and photoperiod (LDN). Fish were maintained at ambient temperature until May 2 when it was increased to either 10, 13, or 16°C or remained at ambient (control).

duction rate (Holmes and Stainer 1966; Eddy and Talbot 1985), and various biochemical and morphological changes (Malikova 1957; Evropeitseva 1960; Fessler and Wagner 1969; Woo et al. 1978). Studies on steelhead trout (*Oncorhynchus mykiss*) (Adams et al. 1973; Zaugg and Wagner 1973), coho (*O. kisutch*) (Zaugg and McLain 1972, 1976), chinook (*O. tshawytscha*) (Zaugg and McLain 1972), and Atlantic salmon smolts (Boeuf and Prunet 1985; Boeuf et al. 1985; Virtanen and Soivio 1985) have associated increases in freshwater temperature above 10°C with a reduction in Na^+, K^+-ATPase activity. The development of hypoosmoregulatory capacity associated with the completion of smoltification in fresh water has been suggested to be accompanied by a disturbance of hyperosmoregulatory mechanisms, as evidenced by net transepithelial efflux of sodium (Primmett et al. 1988), and also a dilution of extracellular fluids can sometimes be observed (Parry 1961; Kubo 1980; Folmar and Dickhoff 1981). Increases in water temperature cause increases in branchial exchange surface area, gill irrigation rate, and cardiac output to compensate for the increased oxygen demand and reduced oxygen availability (Randall 1970), which would exacerbate any existing body fluid dilution problems (Evans 1969; Isaisa 1972). It is hypothesised that the loss of smolt characteristics in salmon prevented from entering saltwater is the result of a corrective homeostatic response to a temperature-accelerated dilution of the body fluids, leading to a loss in hypoosmoregulatory capacity and associated decline in Na^+, K^+-ATPase activity. The present study tests this hypothesis by subjecting smolts to increases in freshwater temperature and quantifying subsequent changes in Na^+, K^+-ATPase activity, blood osmolality, and salinity tolerance.

Materials and Methods

Eyed Atlantic salmon eggs (Saint John River stock) from the Mactaquac Fish Culture Station (New Brunswick) were delivered to St. Andrews on January 16, 1987. The eggs were incubated under constant darkness and 8.0°C until swim-up after which the juvenile salmon were maintained under a simulated natural photoperiod (LDN) cycle (45°N). Temperature was increased to 16.0°C to encourage first feeding and from July until the start of the experiment was ambient, rising to approximately 19°C in mid-August and falling to approximately 3°C in January. On December 3, 1987, fish were graded into potential 1^+-yr-old smolts (fork length $\geq$11.0 cm) and potential 2^+-yr-old smolts (fork length $\leq$11.0 cm). From December 1987 until May 1989 the potential 2^+ smolts selected for the present experiment were maintained in a shallow (water depth = 20 cm) 3-m-square tank supplied with flow-through fresh water (25 $L \cdot min^{-1}$) at ambient temperature and LDN provided by fluorescent light controlled by a time clock adjusted manually every 5 d with no twilight period. Seventy percent of the males became sexually mature in fall 1988, and all fish developed as 2^+-yr-old smolts in spring 1989. On May 2, 1989 (water temperature = approximately 5°C), the fish were randomly divided into four groups (n = 80 per group), each maintained in a lightproof (photoperiod = LDN) 2-m-diameter tank (water depth = 110 cm) supplied with flow-through fresh water (20 $L \cdot min^{-1}$). In three of the groups the water temperature was then raised over a 2- to 3-h period to 10, 13, or 16°C by the addition of hot fresh water controlled by a thermoregulator (accuracy = $\pm$0.2°C). Group 4 (control) remained at ambient temperature (Fig. 1). Due to the natural rise in water temperature the 10°C group was at ambient temperature from May 26 onwards. At regular intervals, a random sample of fish (n = 8) was removed from each group, anaesthetised (1% tertiary amyl alcohol, Fisher), and a blood sample taken from each fish from the caudal vessel using a heparinized syringe. Plasma osmolality was measured using a vapour pressure osmometer (Wescor Inc., 5100c). Also, gill filaments were removed from the first gill arch and frozen in buffer at −80°C until analysed for Na^+, K^+-ATPase activity using the modified method of Zaugg (1982), as described by McCormick et al. (1987), except that gill homogenates were incubated at 20°C rather than 37°C. Enzyme activity was expressed as rate of production of inorganic phosphorus per unit protein (micromoles inorganic phosphate produced per milligram protein per hour). At regular intervals, a random sample (n = 8) from each group was challenged with a tolerance test of 96 h at 37.5 parts per thousand (ppt) salinity). Each group of eight fish was maintained in a translucent plastic box containing 65 L of aerated, recirculating seawater made up to 37.5 ppt with Instant Ocean (Aquarium Systems, Mentor, OH) at the same temperature as the experimental tanks. Boxes were checked at 24-h intervals and dead fish were removed. The number of fish surviving after 96 h was recorded and the salinity tolerance expressed as percent survival. Two-way analysis of variance (ANOVA) followed by one-way ANOVA incorporating Duncan's multiple range test with a significance level set at $P \leq 0.05$ on a SPSS statistical package (Nie et al. 1975) was used to compare inter- and intragroup differences in mean Na^+, K^+-ATPase and plasma osmolality levels. Brownlee's test (Brownlee 1960) was used to compare the percent survival between groups subjected to the tolerance tests.

Results

Gill Na^+, K^+-ATPase Activity

Mean (standard error, SE) Na^+, K^+-ATPase activity increased progressively from 1.8 (0.52) on February 14 to a mean of 6.6 (0.68) on May 2 when the fish were considered to have completed smoltification and the experiment was started (Fig. 2). Three days following the increase in temperature

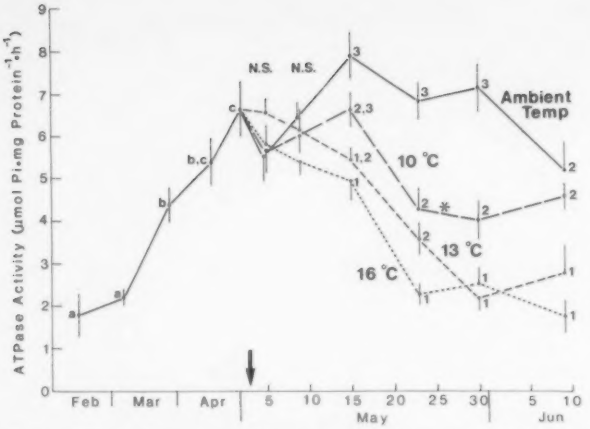


FIG. 2. Effect of experimental temperature regimes on gill Na^+, K^+-ATPase activity (mean ±SE; $n = 8$) of 2⁺-yr-old Atlantic salmon smolts. Letters indicate significant differences ($P \leq 0.05$) from February to the start of the experiment on May 2 (arrow); thereafter, differences between groups at each sampling time are indicated by either N.S. (not significant) or numbers. Means with same letters/numbers are not significantly different. The asterisk indicates when the ambient temperature rose above 10°C.

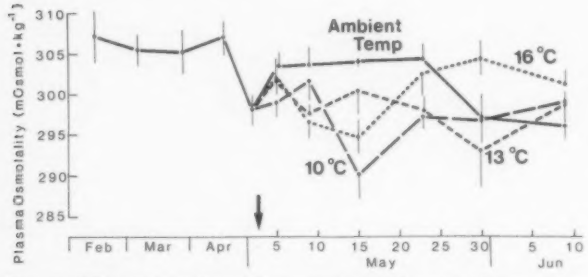


FIG. 3. Effect of experimental temperature regimes on plasma osmolality (mean ±SE; $n = 8$) of 2⁺-yr-old Atlantic salmon smolts. The arrow indicates the start of the experiment.

(May 5), all groups exhibited small, statistically insignificant reduction in Na^+, K^+-ATPase levels (significant differences between groups indicated in Fig. 2). In the control group, mean Na^+, K^+-ATPase activity rose to a peak of 7.9 (0.56) on May 15 declining to 5.2 (0.72) on June 9, but exhibited no statistically significant differences during the experiment. In the 10°C group, mean Na^+, K^+-ATPase activity reached a peak of 6.6 (0.47) on May 15, declining significantly to 4.3 (0.52) on May 23, and thereafter exhibited no significant fluctuation. In the 13°C group, mean Na^+, K^+-ATPase activity decreased from May 2 until May 30, declining significantly between May 15 (5.4 (0.28)) and May 30 to a mean of 2.2 (0.28). In the 16°C group, mean Na^+, K^+-ATPase activity decreased more rapidly than in all other groups, declining significantly between May 2 and May 15 (4.9 (0.45)), reaching 2.3 (0.28) on May 23 and 1.7 (0.42) on June 9.

Plasma Osmolality

Between February 16 and May 2, mean osmolality levels exhibited no significant change, remaining between 298 (2) and 307 (3) mosmol·kg^{-1} (Fig. 3). After the start of the experiment on May 2, there were no significant differences in mean osmolality levels among the four groups, except in samples taken on May 15. On May 15 the group at ambient temperature had a mean osmolality of 306 (1) mosmol·kg^{-1}, which was significantly higher than in the 10°C (290 (3)) and 16°C (295 (2)) groups. ANOVA on osmolality with respect to time revealed that the control group exhibited a significant decline in osmolality between May 23 (304 (2)) and May 30 (297 (3)); the 10°C group exhibited a significant decline in osmolality on May 15 only (290 (3)); in the 16°C group, plasma osmolalities on May 9 (297 (2)) and May 15 (2975 (2)) were significantly lower than on May 30 (304 (2)). The 13°C group exhibited no significant change in osmolality with respect to time.

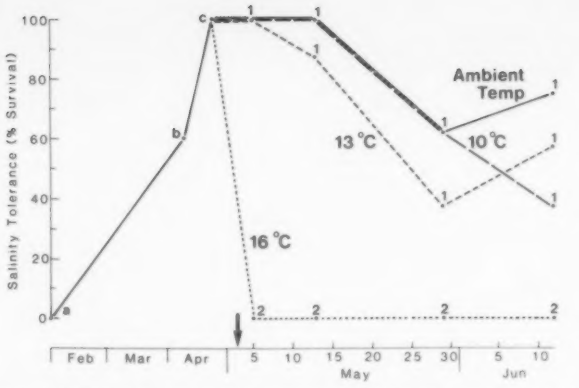


FIG. 4. Effect of experimental temperature regimes on percent survival of 2⁺-yr-old Atlantic salmon smolts ($n = 8$) subjected to 96-h, 37.5-ppt salinity tolerance tests. Letters indicate significant differences ($P \leq 0.05$) from February to the start of the experiment on May 2 (arrow); thereafter, differences between groups at each sampling time are indicated by numbers. Means with same letters/numbers are not significantly different.

Salinity Tolerance Tests

Percent survival in 37.5 ppt for 96 h increased significantly from 0% in February to 62.5% on April 10 and 100% on April 24 (Fig. 4). Following the increases in water temperature on May 2, the 16°C group exhibited 0% survival in all salinity tolerance tests performed at 16°C, which was significantly lower than in all other groups. A single test starting on May 13 using fish from the 16°C group but at an assay temperature of 13°C resulted in 100% survival. No significant differences in percent survival were detected between the ambient, 10°C, or 13°C groups throughout the experiment. On May 13 they exhibited between 87.5 and 100% survival; between May 13 and May 29 the ambient, 10°C, and 13°C groups all showed a significant decline in salinity tolerance. Thereafter no significant changes in salinity tolerance occurred with respect to group or time.

Fish Size

Mean weight (SE) and fork length (SE) of a random sample of 50 fish selected for the experiment were 173.8 g (9.1) and 23.9 cm (0.4) on February 14, 1989, 175.5 (7.6) and 23.8 (0.3) on March 29, increasing significantly to 191.9 (11.1) and 25.3 (0.4) on April 18. After the start of the experiment, fish ($n = 33$–40) were measured on May 19: control group, 184.1 g (12.4) and 26.0 cm (0.5); 10°C group, 193.9, (12.9) and 26.4 (0.5); 13°C group, 180.9 (10.6) and 26.1 (0.4) (all

differences insignificant); the 16°C group was significantly larger than the other groups at 221.2 (17.0) and 27.5 (0.6).

Discussion

The increase in freshwater temperature caused a decrease in gill Na^+, K^+-ATPase activity in Atlantic salmon smolts, with the rate of decrease corresponding to the magnitude of the increase in temperature, similar to previous studies on steelhead trout (Zaugg et al. 1972; Adams et al. 1973) and coho salmon (Zaugg and McLain 1976). These results support the hypothesis that increases in freshwater temperature accelerate the loss of hypoosmoregulatory capacity in smolts. Following the transfer of fish to experimental tanks and increase in freshwater temperature from 5 to 10, 13, or 16°C on May 2, significant differences in mean Na^+, K^+-ATPase levels among groups were observed from May 13 onwards (Fig. 2). In comparison, steelhead trout responded more rapidly to increases in temperature, exhibiting presmolt Na^+, K^+-ATPase activity within 7 d after increasing the temperature from 6.5 to 10°C or above (Adams et al. 1973). In the present study, increases in temperature to 10, 13, and 16°C all resulted in a decrease in Na^+, K^+-ATPase activity compared with controls, suggesting that a threshold temperature for reducing activity of this enzyme existed between 5°C (ambient) and 10°C. However, this threshold temperature for the reduction of branchial Na^+, K^+-ATPase activity in salmonids is not absolute and appears to depend on previous thermal history, similar to the lethal temperature for fish (Brett 1956). For example, the data of Adams et al. (1973) show that 10°C elicited either increases or decreases in Na^+, K^+-ATPase activity depending on whether the previous rearing temperature was higher or lower than 10°C. Also, Atlantic salmon can complete smoltification and exhibit elevated Na^+, K^+-ATPase activity at sustained rearing temperatures of up to 16°C (Johnston and Saunders 1981; J. Duston and R. L. Saunders, unpubl. data). These observations indicate that salmonids can adapt biochemically to different rearing temperatures, possibly by synthesising different isoenzymic forms of the Na^+, K^+-ATPase, as occurs with other salmonid enzymes (Moon and Hochachka 1971), or perhaps by modifying the lipid composition of the cell membrane (Cossins and Prosser 1978) which can also affect Na^+, K^+-ATPase activity (Wallick et al. 1979).

It was proposed that the effect of temperature on the loss of hypoosmoregulatory capacity in smolts was a response to a dilution of the body fluids (see the introduction). However, the stability in plasma osmolality (Fig. 3) refutes this hypothesis and confirms that salmonids have considerable capacity to maintain the iono/osmotic homeostasis of the blood (Reaves et al. 1968; Bath and Eddy 1979; Eddy and Bath 1979; Primmett et al. 1988). Despite these results, it is speculated that smolts retained in fresh water may still be subject to iono/osmotic stress. Body size is of major importance in determining the effect of this stress in salmonids (Parry 1960; Hoar 1976; Jackson 1981), possibly because the surface area to volume ratio is inversely proportional to body weight (Muir 1969). That the fish were large 2^+-yr-old smolts possibly contributed to the maintenance of stable plasma osmolality. In addition, there is evidence that teleosts can maintain the iono/osmotic homeostasis of the blood by using various body compartments as buffers capable of taking up or releasing ions and water (Houston and Threadgold 1963; Houston 1964; Bath and Eddy 1979; Eddy and Bath 1979). This idea is supported by the observation that during the completion of smoltification, Atlantic salmon exhibit an increase in water content (Farmer et al. 1978). Also, Langhorne and Simpson (1986) estimated that smolts retained in fresh water exhibited a concomitant increase in extracellular space and serum cortisol. It is possible that the increase in extracellular space occurs as part of a stress response which helps maintain the iono/osmotic balance of the circulating blood. Further evidence supporting the idea that Atlantic salmon smolts have a reduced hyperosmoregulatory capacity is that dietary intake of salts appears to be important in maintaining ionic balance, since starvation results in a large decline in plasma osmolality from approximately 300 to approximately 240 mosmol·kg^{-1} (J. Duston, D. E. Knox, and P. R. Harmon, unpubl. data), and also a decline in gill Na^+, K^+-ATPase activity has been observed (Virtanen and Soivio 1985). By contrast, salmonids not at the smolt stage can survive long periods of starvation in fresh water with negligible alteration in blood ion concentrations (see Smith et al. 1989).

The effect of temperature on the decline of Na^+, K^+-ATPase activity does not appear to be direct, since in vitro studies have indicated that the optimum temperature for the enzyme in salmonids is approximately 40°C (Giles and Vanstone 1976). A proposed hypothesis to account for the results is that the reduction in activity was mediated indirectly by hormones altering the synthesis and differentiation of gill chloride cells which contain high concentrations of Na^+, K^+-ATPase (Karnaky 1986). The enzyme forms an integral part of the plasma membrane (Hootman and Philpott 1979), and changes in activity are believed to be determined when the immature chloride cells are undergoing differentiation (Foskett et al. 1983). The observed time lag of 9–13 d between increase in temperature and reductions in Na^+, K^+-ATPase activity corresponds well with the estimated turnover rate of 15 d for gill epithelial cells in freshwater-adapted coho salmon at 10°C (Conte and Lin 1967). Endocrine studies on the completion of smoltification in salmonids have implicated such hormones as growth hormone, cortisol, and prolactin in the control of gill Na^+, K^+-ATPase activity (for reviews see Prunet et al. 1989; Young et al. 1989; Boeuf et al. 1990; Madsen 1990), but the mechanism remains unclear and the hormonal changes in association with the loss of smolt characteristics have received little attention. Langhorne and Simpson (1986) recorded an increase in serum cortisol titres in Atlantic salmon following the completion of smoltification; these subsequently declined on transfer of the fish to seawater. They suggested that the rise in cortisol occurred in response to the osmotic stress incurred while smolts, although preadapted to seawater, were held in fresh water and served to protect the fish from suffering iono/osmotic imbalance. By contrast, Young et al. (1989) found that coho salmon smolts exhibited similar increases in plasma cortisol levels whether they were retained in fresh water or transferred to seawater and suggested that hypoosmoregulatory control mechanisms may have evolved differently in coho and Atlantic salmon.

During the completion of smoltification between February and May, gill Na^+, K^+-ATPase activity (Fig. 2) increased concomitantly with salinity tolerance (Fig. 4) in agreement with previous studies (McCormick et al. 1987; Primmett et al. 1988). From May 2 onwards the results of the salinity tolerance tests are confounded by the fact that they were performed at the experimental freshwater temperature to avoid subjecting the fish to a further temperature shock. Zero percent survival in the 16°C group throughout the experiment is at least partly attributable to the 16°C saltwater test temperature. Indeed, a test starting on May 13, and run at 13°C, resulted in 100% survival.

Similarly, brown trout (*Salmo trutta*) transferred in spring directly from fresh water to seawater at 20°C suffered a considerable increase in tissue sodium, while fish transferred to seawater at 10°C did not (Gordon 1959). Despite the variation in salinity tolerance test temperature between the ambient, 10°C, and 13°C groups, there were no significant differences in salinity tolerance, a result which contrasts with the Na^+, K^+-ATPase activity data. The apparent equality in salinity tolerance between the ambient, 10°C, and 13°C groups may be due to the 96-h, 37.5-ppt test not being as sensitive as gill Na^+,K^+-ATPase analysis in detecting changes in hypoosmoregulatory ability. An alternative explanation is that gill Na^+, K^+-ATPase activity as measured in vitro is not always a useful indicator of salinity tolerance (Kirschner 1969; Ewing et al. 1985; Duston et al. 1989; Madsen and Naamansen 1989; Saunders and Harmon 1990) or branchial NaCl excretory ability (Forrest et al. 1973; Scheer and Langford 1976; Madsen and Naamansen 1989), emphasising that firm conclusions concerning the ability of euryhaline fishes to hypoosmoregulate cannot be made on measurement of this enzyme in isolation.

In conclusion, increases in temperature can lead to a decline in Na^+, K^+-ATPase activity in Atlantic salmon smolts retained in fresh water, but the constancy of plasma osmolality refutes the hypothesis that loss of hypoosmoregulatory capacity is a response to dilution of body fluids. However, it is speculated that iono/osmotic stress may still be a reason why smolts retained in fresh water exhibit a decline in hypoosmoregulatory capacity. Smolts retained in fresh water have been described to exhibit "desmoltification" (e.g. Wedemeyer et al. 1980) or "smolt–parr reversion" (e.g. Hoar 1988) implying that all smolt characteristics are lost. However, similarities in growth potential (Saunders and Henderson 1969) and feeding habits (Chernitskiy and Loenko 1983) of Atlantic salmon "post-smolts" in fresh water compared with their seawater counterparts suggest that at least some smolt characteristics are retained in fresh water. Similarly, Gorbman et al. (1982) observed that coho salmon "smolt–parr revertants" retained many of the morphological characteristics associated with a smolt. Thus, it is proposed that complete loss of all smolt characteristics does not occur, and that the generalised terms "desmoltification" and "smolt–parr reversion" can be used only if it is stated what specific aspect of smolt status is being considered.

Acknowledgements

We thank P. Harmon and M. Stewart for technical assistance, Dr. R. Peterson, A. Sreedharan, and the referees for their valuable comments, and B. McMullon and F. Cunningham for preparation of the figures. This work was supported in part by a Canadian Government (Natural Sciences and Engineering Research Council) Postdoctoral Visiting Fellowship to J. Duston and an Industrial Research Assistance Program (IRAP-M) grant to Connors Brothers Ltd. (Aquaculture Division).

References

ADAMS, B. L., W. S. ZAUGG, AND L. R. MCLAIN. 1973. Temperature effect on parr–smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium–potassium stimulated adenosine triphosphatase. Comp. Biochem. Physiol. 44A: 1333–1339.

BAGGERMAN, B. 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (*Oncorhynchus*). J. Fish. Res. Board Can. 17: 295–322.

BATH, R. N., AND F. B. EDDY. 1979. Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh water to seawater. J. Exp. Biol. 83: 193–202.

BOEUF, G., A. LEROUX, J. L. GAIGNON, AND Y. HARACHE. 1985. Gill (Na^+, K^+)-ATPase activity and smolting in Atlantic salmon (*Salmo salar* L.) in France. Aquaculture 45: 73–81.

BOEUF, G., AND P. PRUNET. 1985. Measurements of gill (Na^+, K^+)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon (*Salmo salar*). Aquaculture 45: 111–119.

BOEUF, G., P. PRUNET, AND P. Y. LEBAIL. 1990. Un traitement à l'hormone de croissance peut-il stimuler la smoltification du saumon atlantique ? C. R. Acad. Sci. Paris 310 (Ser. III): 75–80.

BRETT, J. R. 1956. Some principles in the thermal requirements of fish. Q. Rev. Biol. 31: 75–87.

BROWNLEE, K. A. 1960. Statistical theory and methodology of science and engineering. John Wiley & Sons, Inc., New York, NY. 570 p.

CHERNITSKIY, A. G., AND A. A. LOENKO. 1983. The osmoregulatory system and possible ways of differentiation in ecological forms of Atlantic salmon, *Salmo salar*. J. Ichthyol. 23: 84–94.

CONTE, F. P., AND D. H. Y. LIN. 1967. Kinetics of cellular morphogenesis in gill epithelium during seawater adaptation of *Oncorhynchus* (Walbaum). Comp. Biochem. Physiol. 23: 945–957.

CONTE, F. P., AND H. H. WAGNER. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 14: 603–620.

COSSINS, A. R., AND C. L. PROSSER. 1978. Evolutionary adaptation of membranes to temperature. Proc. Natl. Acad. Sci. USA 75: 2040–2043.

DE RENZIS, G., AND M. BORNANCIN. 1984. Ion transport and gill ATPases, p. 65–104. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 10B. Academic Press, New York, NY.

DUSTON, J., AND R. L. SAUNDERS. 1990. The entrainment role of photoperiod on hypoosmoregulatory and growth related aspects of smolting in Atlantic salmon (*S. salar*). Can. J. Zool. 68: 707–715.

DUSTON, J., R. SAUNDERS, P. HARMON, AND D. KNOX. 1989. Increases in photoperiod and temperature in winter advance aspects of smoltification in Atlantic salmon. Bull. Aquat. Assoc. Can. 3: 19–21.

EDDY, F. B., AND R. N. BATH. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri*) adapted to fresh water and dilute seawater. J. Exp. Biol. 83: 181–192.

EDDY, F. B., AND C. TALBOT. 1985. Urine production in smolting Atlantic salmon, *Salmo salar* L. Aquaculture 45: 67–72.

EVANS, D. H. 1969. Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. J. Exp. Biol. 50: 689–703.

EVROPEITSEVA, N. V. 1960. Peculiarities of the desmoltification process as observed in young specimens of various ecological forms of Atlantic salmon. Int. Counc. Explor. Sea, Salmon Trout Comm. 118: 1–11.

EWING, R. D., A. R. HEMMINGSEN, M. D. EVENSON, AND R. L. LINDSAY. 1985. Gill (Na + K)-ATPase activity and plasma thyroxine concentrations do not predict time of release of hatchery coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) for maximum adult returns. Aquaculture 45: 359–373.

FARMER, G. J., J. A. RITTER, AND D. ASHFIELD. 1978. Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 35: 93–100.

FESSLER, J. L., AND H. H. WAGNER. 1969. Some morphological and biochemical changes in steelhead trout during the parr–smolt transformation. J. Fish. Res. Board Can. 26: 2823–2841.

FOLMAR, L. C., AND W. W. DICKHOFF. 1981. Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture 23: 309–324.

FOLMAR, L. C., W. W. DICKHOFF, C. V. W. MAHNKEN, AND F. W. WAKNITZ. 1982. Stunting and parr-reversion during smoltification of coho salmon (*Oncorhynchus kisutch*). Aquaculture 45: 91–104.

FORREST, J. N., JR., A. D. COHEN, D. A. SCHON, AND F. H. EPSTEIN. 1973. Na transport and Na–K-ATPase in gills during adaptation to seawater: effects of cortisol. Am. J. Physiol. 224: 709–713.

FOSKETT, J. K., H. A. BERN, T. E. MACHEN, AND M. CONNER. 1983. Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol. 106: 255–281.

GILES, M. A., AND W. E. VANSTONE. 1976. Changes in ouabain-sensitive adenosine triphosphatase activity in gills of coho salmon (*Oncorhynchus kisutch*) during parr–smolt transformation. J. Fish. Res. Board Can. 33: 54–62.

GORBMAN, A., W. W. DICKHOFF, J. L. MIGHELL, E. F. PRENTICE, AND F. W. WAKNITZ. 1982. Morphological indices of developmental progress in the parr–smolt coho salmon, *Oncorhynchus kisutch*. Aquaculture 28: 1–19.

GORDON, M. S. 1959. Ionic regulation in the brown trout (*Salmo trutta* L.). J. Exp. Biol. 36: 227–252.

HARACHE, Y., G. BOEUF, AND P. LASSERRE. 1980. Osmotic adaptation of *O. kisutch* Walbaum. III. Survival and growth of juvenile coho salmon

transferred to seawater at various times of the year. Aquaculture 19: 253–273.

HOAR, W. S. 1976. Smolt transformation: evolution, behaviour and physiology. J. Fish. Res. Board Can. 33: 1234–1252.

1988. The physiology of smolting salmonids, p. 275–343. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 11B. Academic Press, New York, NY.

HOLMES, W. N., AND I. M. STAINER. 1966. Studies on the renal excretion of electrolytes by the trout (*Salmo gairdneri*). J. Exp. Biol. 44: 33–46.

HOOTMAN, S. R., AND C. W. PHILPOTT. 1979. Ultracytochemical localization of Na^+, K^+-activated ATPase in chloride cells from the gills of a euryhaline teleost. Anat. Rec. 193: 99–130.

HOUSTON, A. H. 1964. On passive features in the osmoregulatory adaptation of anadromous salmonids to seawater. J. Fish. Res. Board Can. 21: 1535–1538.

HOUSTON, A. H., AND L. T. THREADGOLD. 1963. Body fluid regulation in smolting Atlantic salmon. J. Fish. Res. Board Can. 20: 1355–1369.

ISAISA, J. 1972. Comparative effects of temperature on the sodium and water permeabilities of the gills of a stenohaline freshwater fish (*Carassius auratus*) and a stenohaline marine fish (*Serranus scriba, Serranus cabrilla*). J. Exp. Biol. 57: 359–366.

JACKSON, A. J. 1981. Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to seawater. Aquaculture 24: 143–151.

JOHNSTON, C. E., AND R. L. SAUNDERS. 1981. Parr–smolt transformation of yearling Atlantic salmon (*Salmo salar*) at several rearing temperatures. Can. J. Fish. Aquat. Sci. 38: 1189–1198.

KARNAKY, K. J., JR. 1986. Structure and function of the chloride cell of *Fundulus heteroclitus* and other teleosts. Am. Zool. 26: 209–224.

KIRSCHNER, L. B. 1969. ATPase activity in gills of euryhaline fish. Comp. Biochem. Physiol. 29: 871–874.

KUBO, T. 1980. Studies on the life history of the "Masu" salmon (*Oncorhynchus masou*) in Hokkaido. Sci. Rep. Hokkaido Salmon Hatchery 34: 1–95.

LANGHORNE, P., AND T. H. SIMPSON. 1986. The interrelationship of cortisol, gill (Na + K) ATPase, and homeostasis during the parr–smolt transformation of Atlantic salmon (*Salmo salar* L.) Gen. Comp. Endocrinol. 61: 203–213.

MADSEN, S. S. 1990. The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*Salmo trutta trutta*). Gen. Comp. Endocrinol. 79: 1–11.

MADSEN, S. S., AND E. T. NAAMANSEN. 1989. Plasma ionic regulation and gill Na^+, K^+-ATPase changes during rapid transfer to seawater of yearling rainbow trout, *Salmo gairdneri*: time course and seasonal variation. J. Fish. Biol. 34: 829–840.

MALIKOVA, E. M. 1957. Biochemical analysis of young salmon at the time of their transformation to a condition close to the smolt stage, and during retention of smolts in fresh water. Fish. Res. Board Can. Transl. Ser. 232.

MCCORMICK, S. D., AND R. L. SAUNDERS. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. Am. Fish. Soc. Symp. 1: 211–229.

MCCORMICK, S. D., R. L. SAUNDERS, E. B. HENDERSON, AND P. R. HARMON. 1987. Photoperiod control of parr–smolt transformation in Atlantic salmon (*Salmo salar*) : changes in salinity tolerance, gill Na^+, K^+-ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquat. Sci. 44: 1462–1468.

MCINERNEY, H. 1964. Salinity preference: an orientation mechanism in salmon migration. J. Fish. Res. Board Can. 21: 995–1018.

MOON, T. W., AND P. W. HOCHACHKA. 1971. Effect of thermal acclimation on multiple forms of the liver soluble $NADP^+$- linked isocitrate dehydrogenase in the family Salmonidae. Comp. Biochem. Physiol. 40B: 207–213.

MUIR, B. S. 1969. Gill dimensions as a function of fish size. J. Fish. Res. Board Can. 26: 165–170.

NIE, N. H., C. H. HULL, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences. 2nd ed. McGraw-Hill, New York, NY. 675 p.

PARRY, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. J. Exp. Biol. 37: 425–434.

1961. Osmotic and ionic changes in blood and muscle of migrating salmonids. J. Exp. Biol. 38: 411–427.

PRIMMETT, D. R. N., F. B. EDDY, M. S. MILES, C. TALBOT, AND J. E. THORPE. 1988. Transepithelial ion exchange in smolting Atlantic salmon (*Salmo salar* L.) Fish Physiol. Biochem. 5: 181–186.

PRUNET, P., G. BOEUF, J. P. BOLTON, AND G. YOUNG. 1989. Smoltification and seawater adaptation in Atlantic salmon (*Salmo salar* L.): plasma prolactin, growth hormone and thyroid hormones. Gen. Comp. Endocrinol. 74: 355–364.

RANDALL, D. J. 1970. Gas exchange in fish, p. 253–293. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 4. Academic Press, New York, NY.

REAVES, R. S., A. H. HOUSTON, AND J. A. MADDEN. 1968. Environmental temperature and the body fluid system of the fresh-water teleost. II. Ionic regulation in rainbow trout, *Salmo gairdneri*, following abrupt thermal shock. Comp. Biochem. Physiol. 25: 849–860.

SAUNDERS, R. L., AND P. R. HARMON. 1990. Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter–spring. Trans. Am. Fish. Soc. 119: 689–697.

SAUNDERS, R. L., AND E. B. HENDERSON. 1969. Growth of Atlantic salmon smolts and post-smolts in relation to salinity, temperature and diet. Fish. Res. Board Can. Tech. Rep. 149: 27 p.

1978. Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 1542–1546.

SCHEER, B. T., AND R. W. LANGFORD. 1976. Endocrine effects on the cation dependent ATPases of the gills of European eels (*Anguilla anguilla* L.) and efflux of Na. Gen. Comp. Endocrinol. 30: 313–326.

SMITH, N. F., C. TALBOT, AND F. B. EDDY. 1989. Dietary salt intake and its relevance to ionic regulation in freshwater salmonids. J. Fish Biol. 35: 749–753.

VIRTANEN, E., AND A. SOIVIO. 1985. The patterns of T3, T4, cortisol and Na^+K^+ ATPase during smoltification of hatchery-reared *Salmo salar* and comparison with wild smolts. Aquaculture 45: 97–109.

WAGNER, H. H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219–234.

WALLICK, E. T., L. K. LANE, AND A. SCHWARTZ. 1979. Biochemical mechanism of the sodium pump. Annu. Rev. Physiol. 41: 397–411.

WEDEMEYER, G. A., R. L. SAUNDERS, AND W. C. CLARKE. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1–14.

WOO, N. Y. S., H. A. BERN, AND R. S. NISHIOKA. 1978. Changes in body composition associated with smoltification and premature transfer to seawater in coho salmon (*Oncorhynchus kisutch*) and king salmon (*O. tshawytscha*). J. Fish Biol. 13: 421–428.

YOUNG G., B.TH. BJORNSSON, P. PRUNET, R. J. LIN, AND H. A. BERN. 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones, and cortisol. Gen. Comp. Endocrinol 74: 335–345.

ZAUGG, W. S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci. 39: 215–217.

ZAUGG, W. S., B. L. ADAMS, AND L. R. MCLAIN. 1972. Steelhead migration: potential temperature effects as indicated by gill adenosine triphosphatase activities. Science (Wash., DC) 176: 415–416.

ZAUGG, W. S., AND L. R. MCLAIN. 1970. Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt influence in coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 35: 587–596.

1972. Changes in gill adenosinetriphosphatase activity associated with parr–smolt transformation in steelhead trout, coho and spring chinook salmon. J. Fish. Res. Board Can. 29: 167–171.

1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 54A: 419–421.

ZAUGG, W. S., AND H. H. WAGNER. 1973. Gill ATPase activity related to parr–smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comp. Biochem. Physiol. 45B: 955–965.

Chromosome Polymorphism Patterns Indicate Failure of a Scottish Stock of *Salmo salar* Transplanted into a Spanish River

E. García-Vázquez, P. Morán, and A. M. Pendás

Departamento de Biología Funcional (Area Genética), Facultad de Medicina, C/J. Clavería, s/n. 33006-Oviedo, Spain

García-Vázquez, E., P. Morán, and A. M. Pendás. 1991. Chromosome polymorphism patterns indicate failure of a Scottish stock of *Salmo salar* transplanted into a Spanish river. Can. J. Fish. Aquat. Sci. 48: 170–172.

A river in the Asturias region of northern Spain was repopulated with fry originating from two different stocks of *Salmo salar*: native adults and a Scottish stock. A sample of parr captured in the river in 1989 showed the same pattern of chromosome polymorphism as the autochthonous stock released 6 mo before. We conclude that repopulation with the foreign stock was unsuccessful and that chromosome polymorphism patterns yield a useful method of stock discrimination in certain situations.

Une rivière de la région d'Asturias dans le nord de l'Espagne a été ensemencée avec des alevins de *Salmo salar* provenant, d'une part, d'adultes indigènes, et d'autre part, d'un stock écossais. Le polymorphisme chromosomique observé chez des tacons du stock écossais capturés dans cette rivière en 1989 était identique à celui des poissons du stock indigène libérés 6 mo auparavant. L'ensemencement avec le stock étranger s'est donc avéré infructueux. Par ailleurs, il appert que l'analyse du polymorphisme chromosomique constitue une méthode efficace pour discriminer les stocks dans certains contextes.

Received February 27, 1989
Accepted August 16, 1990
(JA072)

Reçu le 27 février 1989
Accepté le 16 août 1990

To preserve variability in natural populations of species exploited by man, a method of genetic characterization of these populations is needed (FAO-PNUMA 1984). Natural populations of the heavily exploited Atlantic salmon, *Salmo salar*, whose most southern distribution is in the rivers of northern Spain, cannot be discriminated in certain situations by conventional means such as isozyme analysis because of the very difficult obtention of large samples. Chromosome polymorphism pattern analysis can be performed in early embryos and requires a small number of individuals (from 30 to 50) for discriminating populations; this is the rationale for choosing this method in very scarce populations such as these analysed here.

In northern Spain, rivers of Asturias are polluted and are subjected to higher fishing pressure than the self-reproducing capacity of their populations can sustain; thus, many populations have decreased or disappeared in the last 20 yr. These rivers have been repopulated since 1969 with fry imported from other countries; from 1986 to 1990, repopulation was carried out also with stocks obtained by spawning of adults captured from other rivers of Asturias (Martín Ventura 1986).

Stocking of Atlantic salmon is a common management strategy in many rivers, but its efficacy is uncertain (Ryman 1970; Bailey and Saunders 1984; Rinne and Minckley 1985; Gjedrem 1986). In the case of the River Esva, we are managing a natural population, not a hatchery one, and intervention carries high risks (Frankel 1983) such as decreasing genetic variation within the population (Vuorinen 1982; Verspoor 1988).

Salmo salar presents intrapopulational chromosome polymorphism (Roberts 1968; Grammeltvedt 1975; Barshene 1978; Hartley and Horne 1984). The number of chromosomes ($2n$) varies from 55 to 60, without variation in the number of arms or fundamental number (NF) (Gold et al. 1980; Hartley and Horne 1984). This chromosomal polymorphism can be used to characterize salmonid populations (Gold and Gall 1975; Thorgaard 1983; Thorgaard and Allen 1987) by considering variability in the number of chromosomes.

Asturian rivers were repopulated with two stocks presenting different chromosome polymorphism (García-Vázquez et al. 1988), one foreign and the other autochthonous. The chromosome distribution of parr that stayed in the river after repopulation may be considered an indicator of the relative adaptation of both stocks to the conditions in the river. If both repopulate adequately, the chromosome distribution found in the parr should depend only on the proportion of individuals released of each stock. Our objective was to assess the efficiency of the repopulation for each stock.

Material and Methods

Karyotypes of 1-mo-old fry of two stocks used to repopulate the River Esva (Asturias, Spain) were studied. One stock from Scotland (Scot-89) was imported as eggs and raised in a hatchery in Asturias. The other (Esva-89) was the result of the spawning of 14 adults (7 females and 7 males) captured in the River Esva. Eggs and fry of both stocks were kept under similar conditions.

The River Esva repopulation was carried out in June 1989, with 6-mo-old fry from both stocks, using 5% Esva fry and 95% Scottish fry. Over 30 000 fry were released. The zone where fry were released and subsequent sampling of parr was carried out has been reported as a natural spawning zone for salmon of this river (Martín Ventura 1986).

In December 1989, a sample of young individuals (parr) was captured by electrofishing in the same place as introduction in June. Karyotypes of 46 individuals (Parr-89) were obtained and

TABLE 1. Number of individuals of each chromosome number. Samples of fry: Esva-89, Scot-89; sample of parr: Parr-89. N.I. = total number of individuals.

	Chromosome number ($2n$)				
Sample	56	57	58	59	N.I.
Esva-89	7	4	39	0	50
Scot-89	0	4	39	7	50
Parr-89	7	9	29	1	46

TABLE 2. Statistical comparisons between the studied samples; chi-square test. Astu = Asturian samples (Esva-89 and Parr-89); N.S. = not significant.

Samples	χ^2 value	df	Statistical significance
Esva-89/Scot-89/Parr-89	22.191	6	$p < 0.01$
Esva-89/Parr-89	2.935	2	N.S.
Astu-89/Scot-89	18.464	3	$p < 0.001$
Scot-89/Parr-89	13.027	2	$p < 0.01$
Esva-89/Scot-89	10.267	2	$p < 0.01$

analyzed. Sampling was carried out over two consecutive days (20 and 26 individuals, respectively). Therefore, the sample may be considered to be representative of that portion of the river, and not a single fraction of parr.

Chromosome preparations of hatchery fry were obtained by the method of Chourrout (1984). The fry were kept swimming for 16 h in a 0.01% colchicine solution. After killing, gills were removed, put in distilled water for 45 min, fixed in 3 parts ethanol to 1 part acetic acid for a minimum of 3 min, and finally dissociated in a drop of 50% acetic acid. The cellular suspension was dropped onto a warmed (50°C) slide, air-dried, and stained in 5% Giemsa for 10 min.

Chromosome preparations of parr were obtained by the following method (developed by Pendás, Morán, and García-Vázquez): fish received an intraperitoneal injection with 0.05 mg of PHA in Hanks solution per 5 g of fish. This procedure was repeated at 24 h. Ten hours later, fish were injected with 0.45 μg of colchicine per gram fish and sacrificed 14 h after colchicination. The anterior kidney was removed, transferred into 4 mL of hypotonic medium (0.56% KCl), homogenized, and kept there for 1.25 h at 20°C. Cells were centrifugated at 1700 rpm for 10 min and fixed in 3 mL of 3:1 methanol/acetic acid for at least 45 min at 4°C. This was repeated three times. After a new centrifugation, cells were resuspended in fresh fixative and dropped onto a warm slide, air-dried, and stained in 5% Giemsa for 15 min. We analyzed at least five metaphases per individual.

Results

Young specimens from the River Esva in 1989 had an estimated age of 1 yr by scale analysis.

Intraindividual polymorphism was found in 0.5% of metaphases. Due to its low proportion, it will not be taken into account when analyzing data.

Chromosome results are presented as the number of individuals of each chromosome type in each analyzed sample and the χ^2 value for statistical differences between samples (Table 1). Parr found in the river had a chromosome distribution similar to the autochthonous fry (Esva-89) and differed from the imported (Scot-89) fry. Always presenting a clear mode in the chromosome number ($2n = 58$), the three samples analyzed had significantly different chromosome distributions (Table 2) ($\chi^2 = 22.191$, 6 df, $p < 0.01$). When partitioning the contingency χ^2, we found that the samples Parr-89 and Scot-89 were different ($\chi^2 = 10.267$, 2 df, $p < 0.01$), but Parr-89 and Esva-89 did not differ at a statistically significant level. We refer to them as Astu-89, that is, Asturian samples. Asturian samples did differ with respect to Scot-89 ($\chi^2 = 18.464$, 3 df, $p<0.001$). Fry from autochthonous adults and those imported were statistically different in 1986, as has been reported in García-Vázquez et al. (1988). We can affirm the same for fry in 1989.

The chromosome number varied in the samples from $2n = 56$ to $2n = 59$, with a cosntant fundamental number (NF) of 74 arms, as in García-Vázquez et al. (1988) this NF is typical of European Atlantic salmon (Hartley and Horne 1984). Cells with $2n = 56$ were found only in Asturian samples.

Discussion

The sample of parr (Parr-89) could be a mixture of those born in the river and hatchery-reared fry because fry used to repopulate the river do remain in the same place (Drummond Sedgwick 1982). Bearing this in mind, we first concluded that in 1989 the parr were not genetically similar to fry released 6 mo before. It must be taken into account that many more Scot-89 fry were used to repopulate the river than Esva-89 and that Scot-89 presented a distribution of chromosome numbers (higher proportion of individuals with $2n = 59$) significantly different from the one of Esva-89. All this indicates that the Scot-89 stock suffered high losses at least in the $2n = 59$ class. Moreover, it is possible that repopulation failed and that parr of 1989 resulted from natural spawning in the river. In this case, selection against hatchery individuals would be immediately after release of the repopulated fry.

If some individuals from Esva-89 and Scot-89 stocks remained, it is clear that there is only one pattern of chromosome polymorphism, indicating that the fry from the Scottish stock have disappeared from the river. This could be due to a decrease in the proportion of $2n = 59$ individuals (independent of their original stock), which would indicate an adaptative role of polymorphism as suggested by Thorgaard (1976, 1983), or to a decrease in the fry from the foreign stock, as Rinne and Minckley (1985) found in Apache trout. Whether or not either mechanism occurs, it seems unquestionable that the population of the river presents its own pattern of chromosome polymorphism that coincides with the pattern of the fry obtained by autochthonous artificial spawning.

Scot-89 and Esva-89 stocks are not equally adequate for repopulation because the chromosome distribution of the population that stays in the river is similar only to Esva-89; this stock is more adequate for repopulation in spite of the fact that it comes from a lower number of parents. Data seem to indicate that the repopulation with foreign fry (or all hatchery fry) is not efficient and it should have been made, in any case, with descendants from the same river in an early stage.

Acknowledgements

We wish to thank Dr. J. Tolivia for his collaboration in analyzing the data. This work was supported by CAICYT 15/84 and by FICYT.

References

BAILEY, J. K., AND R. L. SAUNDERS. 1984. Returns of three year-classes of sea-ranched Atlantic salmon of various river strains and strain crosses. Aquaculture 41: 259–270.

BARSHENE, J. V. 1978. Ontogenetic variability of the chromosome number in the Atlantic salmon (*Salmo salar* L). Genetika 14: 2029–2036.

CHOURROUT, D. 1984. Pressured-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids and heterozygous and homozygous diploid gynogenetics. Aquaculture 36: 111–126.

DRUMMOND SEDGWICK, S. 1982. The salmon handbook. A. Deutsch [ed.] London. p.1–10.

FAO-PNUMA. 1984. Conservación de los recursos genéticos de los peces: problemas y recomendaciones. FAO Doc. Tec. Pesca 217: 42 p.

FRANKEL, O. H. 1983. The place of management in conservation, p. 1–14. *In* C. M. Schonewald-Cox, S. M. Chambers, B. McBryde, and L. Thomas [ed.] Genetics and conservation. The Benjamin Cumming Publ. Co. Inc., Menlo Park, CA.

GARCÍA-VÁZQUEZ, E., A. R. LINDE, G. BLANCO, J. A. SÁNCHEZ, E. VÁZQUEZ AND J. RUBIO. 1988. Chromosome polymorphism in farm fry stocks of Atlantic salmon from Asturias. J. Fish Biol. 33: 581–587.

GJEDREM, T. 1986. Breeding plan for sea-ranching. Aquaculture 57: 77–80.

GOLD, J. R., AND G. A. E. GALL. 1975. Chromosome polymorphism in the California High Sierra golden trout (*Salmo aguabonita*). Can. J. Genet. Cytol. 17: 41–53.

GOLD, J. R., W. J. KAREL, AND M. R. STRAND. 1980. Chromosome formulae of North American fishes. Prog. Fish-Cult. 42: 10–23.

GRAMMELTVEDT, A. F. 1975. Chromosomes of salmon (*Salmo salar*) by leukocyte culture. Aquaculture 5: 1205–1209.

HARTLEY, S. E., AND M. T. HORNE. 1984. Chromosome relationship in the genus *Salmo*. Chromosoma 90: 229–237.

MARTÍN VENTURA, J. A. 1986. Situación del salmón atlántico *Salmo salar* L en los ríos de la Península Ibérica: características de las poblaciones y bases para la recuperación del recurso. Proc. Jornadas sobre la conservación de la naturaleza en España, Oviedo, Spain. p. 139–144.

RINNE, J. N., AND W. L. MINCKLEY. 1985. Patterns of variation and distribution in Apache trout (*Salmo apache*) relative to co-ocurrence with introduced salmonids. Copeia 1985(2): 285–292.

ROBERTS, F. L. 1968. Chromosomal polymorphisms in North American landlocked *Salmo salar*. Can. J. Genet. Cytol. 10: 865–875.

RYMAN, N. 1970. A genetic analysis of recapture frequencies of released young salmon (*Salmo salar*). Hereditas 65: 159–160.

THORGAARD, G. H. 1976. Robertsonian polymorphism and constitutive heterochromatin distribution in chromosomes of the rainbow trout (*Salmo gairdneri*). Cytogenet. Cell. Genet. 17: 174–184.

1983. Chromosomal differences among rainbow trout populations. Copeia 1983: 650–662.

THORGAARD, G. H., AND S. K. ALLEN JR. 1987. Chromosome manipulation and markers in fishery management, p. 319–331. *In* N. Ryman and F. Utter [ed.] Population genetics and fishery management. University of Washington Press, Seattle, WA.

VERSPOOR, E. 1988. Reduced genetic variability in first-generation hatchery populations of Atlantic salmon (*Salmo salar*), Can. J. Fish. Aquat. Sci. 45: 1686–1690.

VUORINEN, J. 1982. Little genetic variation in the Finnish Lake salmon, *Salmo salar sebago* (Girard). Hereditas 97: 189–192.

Long-Term and Short-Term Arsenate Toxicity to Freshwater Phytoplankton and Periphyton in Limnocorrals

Sten-Åke Wängberg

Department of Plant Physiology, University of Göteborg, Carl Skottsbergs gata 22, S-413 19 Göteborg, Sweden

Ulf Heyman

Institution of Limnology, University of Uppsala, Box 557, S-751 22 Uppsala, Sweden

and Hans Blanck

Department of Plant Physiology, University of Göteborg, Carl Skottsbergs gata 22, S-413 19 Göteborg, Sweden

Wängberg, S.-Å., U. Heyman, and H. Blanck. 1991. Long-term and short-term arsenate toxicity to freshwater phytoplankton and periphyton in limnocorrals. Can. J. Fish. Aquat. Sci. 48: 173–182.

A short-term photosynthesis test employing phytoplankton or periphyton communities was a good predictor of the concentration of arsenate that affected these microalgal communities under long-term arsenate exposure in limnocorrals. Arsenate stress changed the structure of the communities to a more tolerant species composition which was consistent with the concept of pollution-induced community tolerance (PICT). The results involving phytoplankton and periphyton in a phosphorus-limited forest lake corroborated earlier findings from the marine environment. Microalgae from the lake were, however, about one order of magnitude more sensitive than the marine communities, probably because these algal communities primarily were limited by phosphorus instead of nitrogen. The observed arsenate toxicity indicated that the natural background of arsenic may already be a structuring factor for the microalgal communities in several freshwater environments.

Un test de photosynthèse de courte durée s'est avéré un bon indicateur de la concentration d'arsenic dans des communautés de microalgues (phytoplancton ou périphyton) exposées à long terme à cette substance. L'exposition à l'arsenic a eu pour effet de modifier la structure des communautés : les espèces les plus tolérantes en sont venues à dominer la population, ce qui corrobore l'idée selon laquelle les communautés développeraient une tolérance à la pollution. Les résultats concernant le phytoplancton et le périphyton dans un lac forestier où leur croissance est limitée par la teneur en phosphore sont en accord avec d'autres résultats obtenus en milieu marin. Dans le lac, les microalgues étaient cependant environ 10 fois plus sensibles que les algues du milieu marin, probablement parce que leur croissance était d'abord limitée par le phosphore plutôt que par l'azote. La toxicité observée de l'arsenic montre que la nature du milieu où il se trouve peut être en soi un facteur intervenant dans la structuration des communautés d'algues dans plusieurs milieux d'eau douce.

Received February 16, 1989
Accepted August 20, 1990
(JA058)

Reçu le 16 février 1989
Accepté le 20 août 1990

A microalgal test system in which effects of toxicants on community metabolism were measured in short-term experiments was developed by Blanck (1985). The test system was designed to combine the community-level approach with a high test capacity. Validation of the test with marine periphyton communities showed a good agreement between the arsenate concentration which reduced photosynthesis in the short-term test and that which changed the structure of communities after long-term exposure (Blanck and Wängberg 1988a).

Based on this test system, a new ecotoxicological tool, pollution-induced community tolerance (PICT) (Blanck et al. 1988), was introduced to detect whether or not a toxicant has affected a community. The hypothesis is that a community exposed to a toxicant at sufficiently high concentrations would change its structure and community tolerance because organisms sensitive to the toxicant would have a decreased competitive ability and would be suppressed by organisms with higher tolerance. An increased community tolerance would therefore indicate that a community had been affected by the toxicant. In a model ecosystem, PICT correctly detected that 0.32 μM arsenate, but not 0.1 μM, had an impact on marine periphyton communities (Blanck and Wängberg 1988b).

The close correlation between long-term changes in marine periphyton communities and short-term inhibition of carbon dioxide fixation (Blanck and Wängberg 1988a) suggests that carbon dioxide fixation, or a related process, is ecologically the most important target for arsenate in microalgae. One physiological mechanism for inhibition of carbon dioxide fixation by arsenate is by a competitive inhibition of photophosphorylation as shown with isolated chloroplasts by Avron and Jagendorf (1959). Furthermore, the cotolerance pattern for arsenate-tolerant periphyton suggests that the mode of action of arsenate in periphyton is by competition with phosphate in photophosphorylation (Blanck et al. 1988).

The influence of phosphate concentration on the toxic effects of arsenate in microalgae is well documented (Planas and Healey 1978; Sanders 1979; Wängberg and Blanck 1990). One should therefore expect differences in arsenate toxicity in waters

where primary production is limited by phosphorus and nitrogen, respectively.

To extend the studies of arsenate effects to phosphorus-limited freshwater systems would thus improve the validation of the short-term test system and the PICT hypothesis. This study aims to (1) evaluate the ability of the short-term test to predict the long-term effects of arsenate on freshwater phytoplankton communities, (2) evaluate the ability of PICT to indicate long-term effects of arsenate on freshwater microalgal communities, and (3) contribute to the hazard assessment of arsenate in freshwater ecosystems.

Material and Methods

Experimental Outline

This work fell into two parts. The first dealt with the short-term effects of arsenate on photosynthesis of algal communities, and the second with the long-term effects of algal community structure and biomass in arsenate-loaded limnocorrals. The short-term tests using communities from the lake and control corral were used to predict long-term effects of arsenate (Fig. 1). This prediction was tested against changes in algal community structure and biomass in arsenate-loaded corrals. The long-term effects were also detected as an increased arsenate tolerance (PICT), using the short-term test to quantify the tolerance levels in the various corrals.

Corrals, Arsenate Additions, and Nutrient Conditions

The detailed design of the limnocorral experiment was given by Landner et al. (1989). Four limnocorrals were placed in Lake Sörmogen, a mesohumic and mesotrophic forest lake in central Sweden, with an area of 3.7 km^2. The corrals had glassfibre-reinforced polyethylene walls and were 10 m in diameter and 4 m in depth with an approximate volume of 300 m^3. The enclosures were open to the sediment into which the walls were sunk by means of 15-mm-thick iron bars sewn into the plastic. Peristaltic pumps were used to continuously pump water in and out of the corrals, yielding a theoretical turnover time of 100 d. The corrals were installed on May 23, 1986, about 2 wk after the ice breakup in the lake. Continuous addition of sodium arsenate started 2 wk later. During 3 d the arsenate concentrations were raised to the desired nominal arsenate levels of 270 nM ("high"), 135 nM ("medium"), and 35 nM ("low") above background (16 nM). One corral without any arsenate addition acted as control. After the initial dose of arsenate, additions were made to the water pumped into the corrals to maintain the nominal levels. However, during summer, most added arsenic disappeared from the water column in the corrals. Therefore, on September 9, arsenate was again added to raise the concentrations to the nominal levels. The measured arsenic concentrations in all corrals and the lake during the whole experiment are given in Fig. 2. On the average, 74% of the arsenic in the lake was dissolved (passing a 0.2-μm Gelman filter). At elevated arsenic concentrations in the corrals, higher percentages were dissolved.

The ratio between total nitrogen (N/P) (calculated as nitrate-N + Kjeldahl-N) and total phosphorus indicated phosphorus limitation of the primary production. The lowest N/P value was 48 (mol/mol) and the highest 104. The ratio between total phosphorus and total arsenic in lake water varied between 22 and 39 (mol/mol) with a mean of 30.

Sampling and Analysis of Water and Algal Communities

Phytoplankton and water in the corrals and the lake were sampled on 20 occasions during the experiment with a 2-m tube sampler of 2 L volume. From each of the corrals and from the lake, composite samples were taken using four surface (0–2 m) and four bottom (2–4 m) samples. Each 16-L phytoplankton sample was subsampled and preserved with acidified Lugol's solution (IIK). After sedimentation of 10 mL of the IIK-preserved subsample, phytoplankton were counted using an inverted microscope according to Utermöhl (1958). Usually 100–500 units (cells, colonies, or filaments) of the most common taxa were counted in each sample. Phytoplankton cell volumes were obtained using geometric formulas (Blomqvist et al. 1981).

On two occasions (August 20 and October 3), phytoplankton were sampled for short-term tests. The time from sampling to start of the photosynthesis measurements was less than 1 h. Samples were transported to the laboratory in 1-L Pyrex glass bottles.

Periphyton was also sampled (on October 3) for short-term tests and biomass determination. Sampling units for periphyton communities were glass discs with an area of 1.5 cm^2 placed in a frame, with 17 disc holders each carrying 10 discs (Blanck and Wängberg 1988a). The frames were placed at a depth of 0.5 m in the centre of the corrals and in the lake. Before the test, 10 disc holders were transported to the laboratory in a polyethylene container filled with lake water. A set of discs similar in periphyton density as judged by gross appearance was selected to ascertain a similar biomass and photosynthetic activity in the replicate samples. The time from sampling to start of the experiment was less than 1 h.

Chlorophyll in periphyton and filtered (Whatman GF/F) phytoplankton samples was extracted with dimethysulphoxide and analysed spectrophotometrically (Blanck and Wängberg 1988a). Total carbon and nitrogen in the periphyton were analysed in communities scraped from the glass discs (Blanck 1985). Total arsenic was determined with hydride generation flameless absorption spectrometry (Borg et al. 1985). Prior to these measurements, organic arsenic was oxidized with UV irradiation and addition of hydrogen peroxide (water samples), or with dry ashing under acidic conditions (periphyton). After oxidation, arsenate was reduced to arsenite through addition of concentrated HCl and 10% KI solution. Hydrides were generated by addition of HCl (6 M) and sodiumborohydride (1% w/v). The generated hybrides were swept by argon into an electrically heated (850°C) quartz tube mounted in the light path of an atomic absorption spectrometer. In the reduced solutions from the arsenic analysis, total phosphorus was also determined using a molybdate reagent (Koroleff 1976). By this procedure, interference of arsenate on the phosphate determination was avoided. Nitrate-N and Kjeldahl-N of the water were analysed according to Rand et al. (1976).

Photosynthesis Experiments

Photosynthesis was measured with ^{14}C technique. Phytoplankton was incubated in scintillation vials each containing 4.5 mL of phytoplankton (unfiltered water from the lake or the corrals) and 0.5-mL test solutions of arsenate, dissolved in filtered (Whatman GF/F) lake water, to give the desired arsenate concentrations. Test solutions were prepared the evening before the experiments and stored at 5°C. To test whether dissolved substances (e.g. humus) in the filtered lake water reduced the

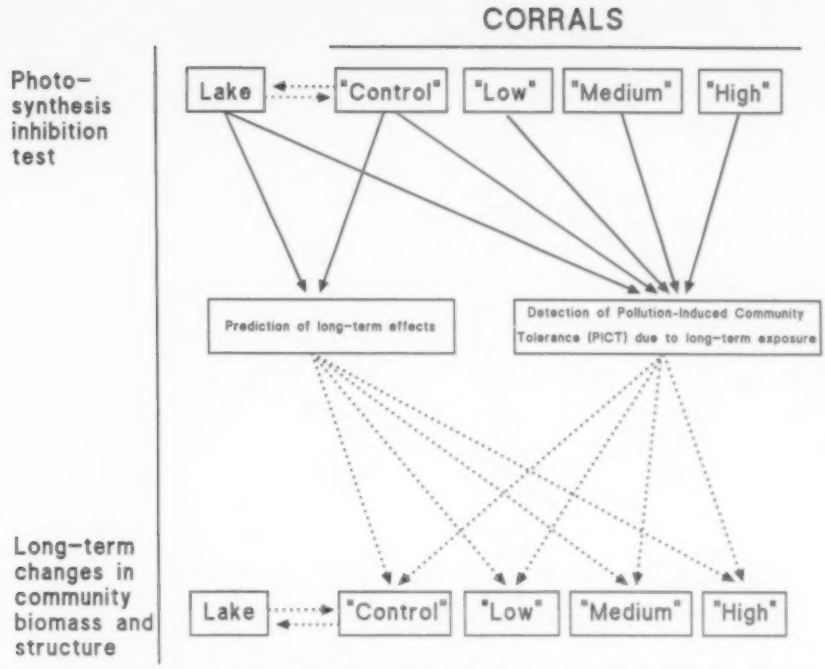


FIG. 1. General outline for the experiments. The central boxes represent conclusions (predictions and detections) from the short-term tests. Solid lines indicate the communities used for the different conclusions, while broken lines indicate validations of these conclusions in long-term tests. Broken lines are also drawn to indicate comparisons between the phytoplankton communities in the control corral and in the lake, respectively.

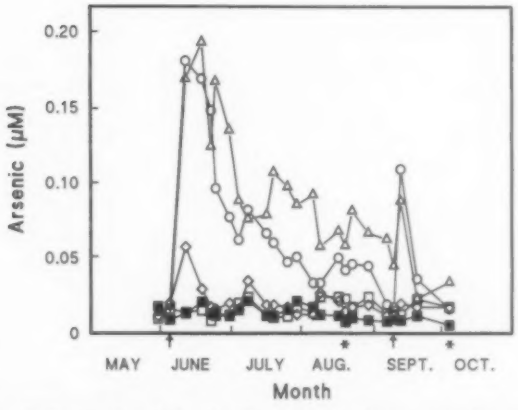


FIG. 2. Seasonal variation in dissolved arsenic concentration of the lake and the corrals. Arrows indicate the start of arsenate addition in June and the extra arsenate addition in September. Asterisks indicate the time for the short-term tests. □, lake; ■, control corral; ◇, "low" corral; ○, "medium" corral; △, "high" corral.

toxicity of added arsenate, an extra arsenate solution was prepared with distilled water. In this experiment the vials contained 4.9 mL of phytoplankton and 0.1 mL of arsenate – distilled water solution. The periphyton samples were incubated in liquid scintillation vials each containing 5 mL of test solution based on filtered lake water with additions of arsenate.

A geometric, nominal, concentration series (factor 2.1 or 10) of arsenate from 1 nM to 1 mM was tested. With the lower factor we used a single sample from each concentration, while we used three replicates from each arsenate concentration made with a factor of 10. Five replicates were always used for the control. The samples were stirred by gentle shaking during incubation. The temperature in the incubator was regulated by thermostat to be the same as that in the lake (about 10°C). Light was provided by fluorescent tubes (Osram Lumilux Daylight L18W/11). The light was filtered through 10 cm of water, giving an irradiance, during incubation, of 125 μE/(m^2·s). After a 1-h preincubation period in dim light, a 50-μL (2 μCi, 74 kBq) aliquot of [^{14}C]bicarbonate dissolved in NaOH solution (pH 9) was added to each vial. After the incubation period, photosynthesis was stopped by adding 200 μL of formaldehyde solution (20%). The incubation period was 15 min for periphyton, while we used 30 min for phytoplankton due to the lower biomass. Phytoplankton samples were acified with HCl and bubbled with air. Ten millilitres of scintillation cocktail (Instagel, Packard Instruments Co.) was then added and the samples were thoroughly mixed. In the periphyton experiments the test solutions were removed with a suction pump; the remainder was acidified with acetic acid and dried at 60°C. The dried samples were dissolved by adding 0.3 mL of distilled water and 1 mL of tissue solubilizer (Soluene 350, Packard Instrument Company Inc.). Ten millilitres of scintillation cocktail (Omnifluor, New England Inc., in toluene) was added and the samples thoroughly mixed. A Packard Tricarb liquid scintillation spectrometer 3255, using channel ratio for quench correction, was used to determine the incorporated ^{14}C. The ^{14}C incorporated into samples that were killed with formaldehyde

TABLE 1. EC_{50} values for inhibition of photosynthesis by arsenate in phytoplankton and periphyton communities. n.d. = not determined.

Corral	EC_{50} (μM)	
	Phytoplankton	Periphyton
August		
Control	0.58	n.d.
"Low"	0.15	n.d.
"Medium"	0.65	n.d.
"High"	5.67	n.d.
Lake, August 19	0.58	n.d.
Lake, August 20	0.18	n.d.
October		
Control	0.13	0.02
"Low"	n.d.	0.04
"Medium"	0.07	0.02
"High"	243.00	0.34
Lake	0.08	0.13

before the addition of [^{14}C]bicarbonate was subtracted from all readings.

Mathematical and Statistical Methods

The inhibition values of carbon dioxide fixation by arsenate in the different communities were compared by Wilcoxon matched-pairs signed-ranks test (Siegel 1956), when each arsenate concentration was represented by a single sample. When three replicates were used for each arsenate concentration, a two-way ANOVA test was used. A logarithmic transformation of the photosynthetic activities was used in the analysis to achieve homogeneous variances. A difference in activity between 3 and 6% will thus have the same weight as a difference between 50 and 100%. Because we wanted to limit the comparison to the major part of the photosynthetic activity, we did not include periphyton concentrations over 1 μM. They all had photosynthetic activities that were less than 10% of the control. EC_{50} values (the concentration of arsenate needed to inhibit photosynthesis by 50%) were calculated by geometric interpolation.

The degree of similarities in taxa composition of the different phytoplankton communities was estimated using a principal component method, SIMCA (Wold et al. 1983), and the dissimilarity form of the Czekanowski index, CDI (Greig-Smith 1983), which is defined as

$$(1)\quad \mathrm{CDI} = \frac{\sum_{i=1}^{m} |n_{1i} - n_{2i}|}{\sum_{i=1}^{m} (n_{1i} + n_{2i})}$$

where n_{i1} and n_{i2} is the biomass of the ith algal taxon in the two communities (1 and 2), respectively, and m is the total number of taxa observed in any of the two communities. The SIMCA software is available from Sepanova AB, Enskede, Sweden.

Similarities in taxa composition were always calculated using, as input, the cell volume of each algal taxon as a percentage of the total algal cell volume. Calculations were made both on the original data and on data transformed to the fourth square. The transformation gives relatively more weight to the less abundant taxa (Clark and Green 1988).

TABLE 2. Coefficient of variation in photosynthetic activity at background arsenate concentration. The coefficient is calculated as the ratio between the mean and the standard deviation of the photosynthesis measurements (n = 5) in samples without any added arsenate. n.d. = not determined.

Corral	Coefficient of variation		
	Phytoplankton		Periphyton, October
	August	October	
Control	0.30	0.077	0.12
"Low"	0.26	n.d.	0.30
"Medium"	0.29 (n=4)	0.082	0.46
"High"	0.25	0.035	0.59
Lake	0.31	0.150	0.15

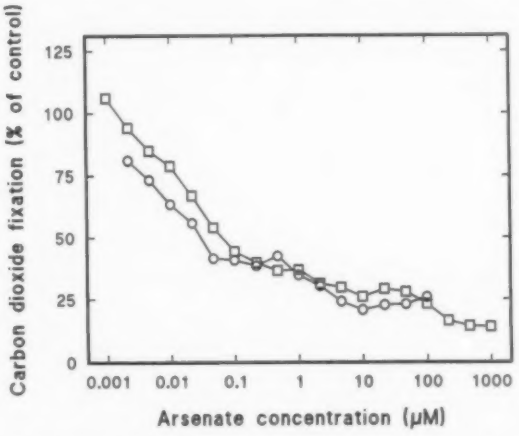


FIG. 3. Inhibition of carbon dioxide fixation in phytoplankton by arsenate solutions prepared in filtered lake water and distilled water. A single sample was tested for each arsenate concentration, while five replicates were used for the control. The curves were smoothed by taking moving averages over three samples. □, lake water; ○, distilled water.

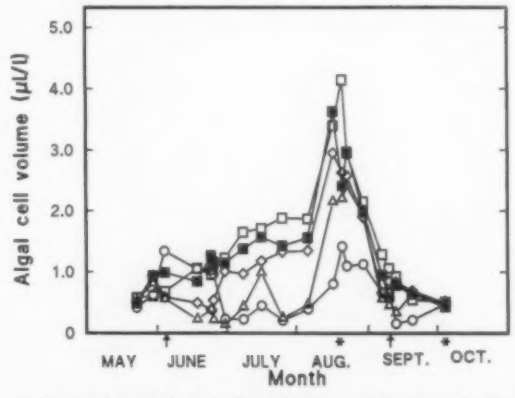


FIG. 4. Seasonal variation in phytoplankton cell volume in the lake and the corrals. The curves were smoothed by taking moving averages over three samples. Arrows indicate the start of arsenate addition in June and the extra arsenate addition in September. Asterisks indicate the time for the photosynthesis experiments. □, lake; ■, control corral; ◇, "low" corral; ○, "medium" corral; △, "high" corral.

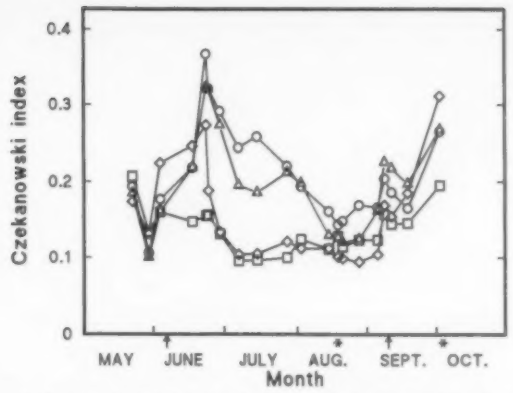


FIG. 5. Seasonal variation in algal taxonomic composition between the control community and other communities. The difference in taxonomic composition was expressed as the Czekanowski dissimilarity index. For each taxon, its part of the total algal cell volume transformed to the fourth square root was used as input. Before transformation the relative abundances were smoothed by taking moving averages over three samples. Arrows indicate the start of arsenate addition in June and the extra arsenate addition in September. Asterisks indicate the time for the photosynthesis experiments. □, lake; ◇, "low" corral; ○, "medium" corral; △, "high" corral.

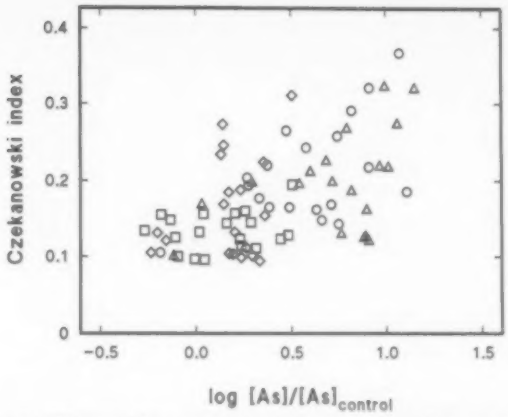


FIG. 6. Difference in community structure as dependent on arsenic concentration. The difference in structure was expressed as the Czekanowski dissimilarity index when compared with the control corral. For each taxon, its part of the total algal cell volume transformed to the fourth square root was used as input. Before transformation the relative abundances were smoothed as in Fig. 2. Arsenic concentration differences are given as the logarithm of the ratio between the arsenic concentration in the corral and that in the control corral. □, lake; ◇, "low" corral; ○, "medium" corral; △, "high" corral.

Results

Short-Term Effects of Arsenate on Photosynthesis

Unexposed periphyton and phytoplankton communities in the lake and control corral were, on October 3, very sensitive to arsenate. The EC_{50} values for inhibition of photosynthesis ranged from 0.02 to 0.13 μM (Table 1). Phytoplankton communities from the same sources tested on August 20 appeared more tolerant, with EC_{50} values up to 0.58 μM. The variance was, however, greater in August (Table 2), increasing the uncertainty in the EC_{50} estimates. For example, despite a large difference in EC_{50} values (0.58 and 0.18 μM) of lake phytoplankton measured on consecutive days in August, the difference between two dose–response curves was not statistically significant (two-way ANOVA test).

The effect of lake water on arsenate toxicity was determined by comparison with the toxicity of arsenate dissolved in distilled water. The latter arsenate solution was significantly more toxic ($P < 0.05$) (Fig. 3), indicating that overnight storage of arsenate in GF/F-filtered lake water decreased the toxicity/availability of arsenate to phytoplankton.

Long-Term Effects of Arsenate on Phytoplankton Cell Volume and Structure

Effects on algal cell volume

The total algal cell volume decreased in all the arsenate-loaded corrals when compared with the control corral and the lake (Fig. 4). The increase in arsenic concentration required to decrease the algal cell volume (the no-effect concentration) was thus equal to or less than the highest concentration in the "low" corral (59 nM) minus the background concentration (16 nM) which gives 43 nM, which is close to the nominal concentration of 35 nM. During the initial input of arsenate the concentration might locally have been higher although the gradual addition of arsenate over 3 d should have minimized that risk. The phytoplankton of the "low" corral recovered to the same cell volume as the control when the arsenic concentration decreased in late June, while the "medium" and "high" corrals had lowered phytoplankton cell volume until late August. No changes in algal cell volume could be correlated with the increase in arsenate concentration in early September. However, the short duration of the increased arsenate concentration and the fact that it came right at the end of a bloom by *Gonyostomum semen* might have masked such effects.

Effects on toxonomic composition

Increased arsenic concentrations were correlated with changes in phytoplankton taxonomic composition as judged from Czekanowski's dissimilarity index (Fig. 5). A significant ($P < 0.001$, Spearman rank test) correlation was found between the ratio in arsenic concentration between the arsenic-treated corrals and the control and the Czekanowski dissimilarity index (Fig. 6). The seasonal changes were, however, more important than the arsenic load as structuring factors which is seen in the principal components plot calculated with SIMCA (Fig. 7). The Czekanowski index and principal components presented are based on abundances transformed to the fourth square root to increase the influence of less frequent taxa on the models. The transformation did not introduce any qualitative changes in the values.

Pollution-Induced Community Tolerance (PICT)

The PICT hypothesis was tested for three sets of communities coming from the corrals: phytoplankton in August and October and periphyton in October. In these three cases the communities from the "high" corral were always the most tolerant (Table 3; Fig. 8). The dose–response curves from the "high" corral were significantly different from the others in almost all comparisons (Table 3). The only exceptions were for some phytoplankton communities in August when the variance in photosynthesis measurements was high. Significant differences in dose–response curves between other pairs of communities were rare.

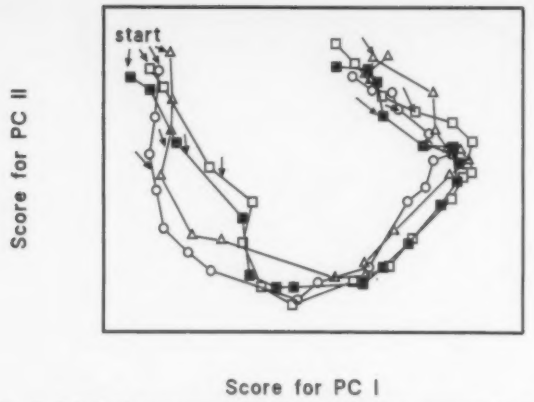


FIG. 7. Principal component plot of phytoplankton community structure. Components were calculated by SIMCA using the percentage of total algal cell volume for each algal taxa in all communities as input transformed to the fourth square root. The different algal taxa were used as variables and communities as objects. A principal component is the vector that explains most of the variation in a hypervolume. The plot can thus be seen as a mirroring of the comunities on that plane in the hypervolume that explains most of the variation in abundance of taxa. Both components were significant as judged by cross-validation. The lines connect the communities from the same location in chronological order. The approximate times for arsenate additions are indicated by arrows on the lines. □, lake; ■, control coral; ○, "medium" corral; △, "high" corral.

TABLE 3. Significance of statistical differences in sensitivity to arsenate between microalgal communities. For periphyton and phytoplankton from August, differences in inhibition of photosynthesis were estimated by a two-way ANOVA test. In a few cases, data were missing, so to achieve equal number of samples in each cell for statistical analysis, data were deleted at random from cells with a surplus. In the October experiments with phytoplankton, differences were estimated by a Wilcoxon matched-pairs signed-ranks test. n.d. = not determined; n.s. = not significant; *$0.02<P<0.05$; **$0.01>P>0.02$; ***$P<0.01$.

Community	Control	"Low"	"Medium"	"High"
Lake				
Phytoplankton, August	n.s.	n.s.	n.s.	**
Phytoplankton, October	n.s.	n.d.	n.s.	***
Periphyton, October	***	n.s.	n.s.	***
Control				
Phytoplankton, August		n.s.	n.s.	n.s.
Phytoplankton, October		n.d.	*	***
Periphyton, October		n.s.	n.s.	***
"Low"				
Phytoplankton, August			n.s.	***
Periphyton, October			n.s.	***
"Medium"				
Phytoplankton, August				n.s.
Phytoplankton, October				***
Periphyton, October				***

According to the PICT concept, changes in community structure due to exposure to a toxicant are manifested as an increased community tolerance. The structure of communities from the "high" corral at the time of the PICT tests is therefore important.

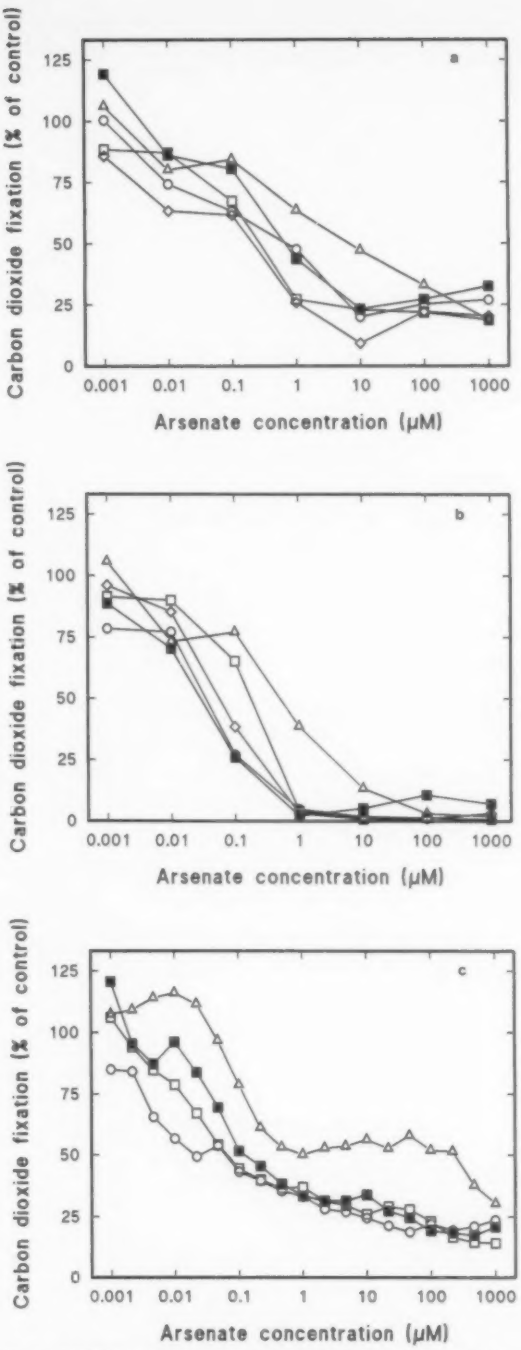


FIG. 8. Inhibition of carbon dioxide fixation by arsenate in microalgal communities. The mean activities of carbon dioxide fixation expressed as a percentage of the control ($n = 5$) are given. (a) Phytoplankton communities from August 20. Three replicates were used for each arsenate concentration. (b) Periphyton communities from October 3. Three replicates were used for each arsenate concentration. (c) Phytoplankton communities from October 4. A single sample was used for each arsenate concentration and the curves were smoothed taking moving averages over three samples. □, lake; ■, control corral; ◇, "low" corral; ○, "medium" corral; △, "high" corral.

TABLE 4. Taxa in the tested phytoplankton communities that showed extreme abundance (either the highest or the lowest) in the "high" corral either in August or October. Algal taxa with >1 and >5% of the total algal cell volume are included in the August (a) and October (b) tables, respectively. These percentages correspond to about 5 nL/L. Taxa with extreme (either the highest or the lowest) abundances in the tolerant "high" corral are indicated with an asterisk. The number of recorded taxa in each community is also given.

Algal taxa	% of total algal cell volume				
	Lake	Control	"Low"	"Medium"	"High"
(a) Phytoplankton communities from August 20					
Chrysophyceae					
Ochromonadaceae					
Spp. No. 1 (3–4 μm)	1.11	1.99	1.06	3.16	1.82
Spp. No. 3 (8–10 μm)*	0.56	0.64	0.72	0	1.23
Bacillariophyceae					
Melosira ambigua	0.72	3.23	2.09	1.34	1.23
Cyclotella spp.	0.68	2.61	0.27	0.99	0.64
Rhizosolenia longiseta	1.01	0.54	0.68	0	0.14
Tabellaria fenestrata	13.7	14.8	14.6	6.33	9.90
*Diatoma elongata**	18.5	19.9	21.0	10.7	7.93
Chlorophyceae					
*Polytoma granuliferum**	0.63	0.87	0.19	1.12	0
Cryptophyceae					
Cryptomonas spp.*					
(e.g. tetrapyrenoidosa)	1.25	2.36	1.25	2.18	0.46
Chloromonadophyceae					
*Gonyostomum semen**	46.0	31.9	39.2	54.0	61.1
Dinophyceae					
*Gymnodinium uberrimum**	1.40	1.33	1.14	1.12	0.55
*Peridinium inconspicuum**	0.29	1.83	2.05	0.70	0.09
Number of recorded taxa	48	46	49	44	44
(b) Phytoplankton communities from October 3					
Chrysophyceae					
Ochromonadaceae					
Spp. No. 1 (3–4 μm)*	2.76	3.73	1.6	5.04	5.37
Spp. No. 3 (8–10 μm)	0	0	5.3	0	1.59
Uroglena sp.*	1.97	3.03	1	3.36	13.32
Bacillariophyceae					
*Melosira ambigua**	30.97	25.64	20.2	19.32	14.31
Cyclotella spp.*	0	1.63	2.9	0	8.35
*Rhizosolenia longiseta**	15.58	13.52	17.4	11.13	5.17
*Tabellaria fenestrata**	10.45	9.32	8.4	10.30	6.96
Dinophyceae					
Peridinium inconspicuum	7.10	4.20	17.1	8.82	9.54
Number of recorded taxa	31	35	31	38	38

Specific Structures of the Tolerant Communities

The number of recorded taxa in the "high" corral showed no difference from the other corrals either in August or October (Table 4); neither did the small differences in the Czekanowski index (Fig. 5) at the test occasions indicate that the communities from the "high" corral were structurally different. When, however, single taxa were analysed, similarities were found between the abundance in the PICT-tested communities and the clearly affected communities in "high" and "medium" corrals.

Thirteen taxa (Table 4) had either a higher or a lower abundance in the "high" corral than in any of the other communities when results from both test occasions were considered. Eight of these taxa differed significantly ($P < 0.05$, Mann–Whitney U-test) in their abundances in the control and in the exposed communities during the period June to mid-August (Fig. 9).

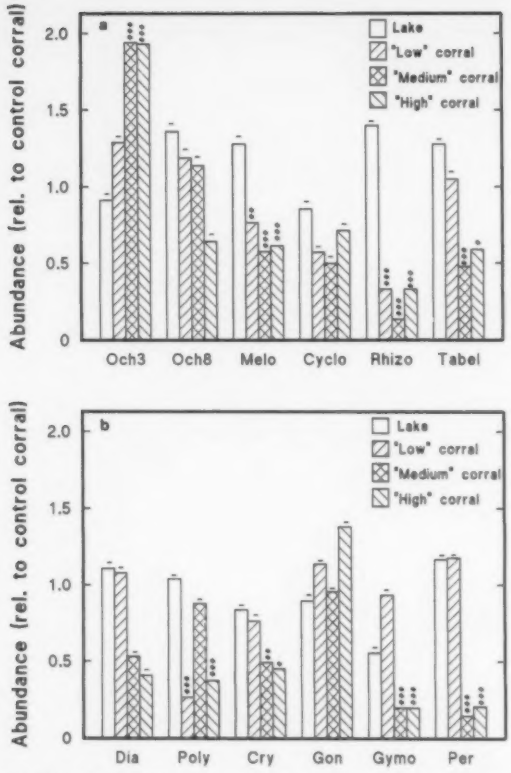


FIG. 9. Relative abundances of different algal taxa in the arsenate-loaded corrals and the lake relative to the control corral during mid-June to mid-August. The cell volume of each taxon was divided by the total algal cell volume for each sample from the start of arsenate addition to mid-August (total of 11 samples from each corral). These ratios are given after normalization to the corresponding ratio in the control corral. – = $P > 0.05$; *$P < 0.05$; **$P < 0.025$; ***$P < 0.01$. (Differences in ratios between the control and the treated corrals and lake were tested by a one-tailed Mann–Whitney U-test.) (a) Och3 = Ochromonadaceae sp. No. 1 (3–4 μm); Och8 = Ochromonadaceae sp. No. 3 (8–10 μm); Melo = *Melosira ambigual*; Cyclo = *Cyclotella* spp.; Rhizo = *Rhizosolenia longiseta*; Tabel = *Tabellaria fenestrata*. (b) Dia = *Diatoma elongata*; Poly = *Polytoma granuliferum*; Cry = *Cryptomonas* spp. (e.g. tetrapyrenoidosa); Gon = *Gonyostomum semen*; Gymu = *Gymnodinium uberrimum*; Per = *Peridinium inconspicuum*.

All these differences were in the same direction as for the tested communities from the "high" corral. The other four taxa showed no significant ($P > 0.05$) differences in abundance from the control. (*Uroglena* sp. was not included in the comparison, as it was not found during June to mid-August.) The differences in abundance between the more tolerant communities and the others thus resembled the differences observed during summer between the clearly affected communities and the others.

Discussion

Artificial enclosures such as limnocorrals differ from entire ecosystems in several respects. Important components (primarily top consumers like fish) are missing, water movements are different, new habitats are formed on the walls, etc. The practice of using corals as substitute ecosystems in experimental ecology has therefore been criticized (e.g. Schindler 1987). Some typical wall effects such as increased abundance of zooplankton (Bell and U. Heyman, pers. comm.) and tychoplanktonic algae (Blomqvist and Heyman 1989) were seen also in our corrals. However, the similarity of phytoplankton between the lake and the control corral in both cell volume (Fig. 4) and taxonomic composition (Fig. 5) justifies the use of these corrals for validating ecotoxicological tools and extrapolating the results to the ecosystem level.

Validation of the Short-Term Test

An estimate of the sensitivity to arsenate over the long term, based either on effect on cell volume (Fig. 4) or taxonomic composition (Fig. 5), showed that as little as 43 nM arsenate (or two to three times the background) induces changes in the algal communities. The EC_{50} values from October are between 20 and 130 nM (Table 1) which suggests that the short-term test correctly estimates the long-term toxicity of arsenate within a factor of 6, the same factor as we found earlier for marine periphyton communities (Blanck and Wängberg 1988a). These factors should be compared with the EC_{100} values, for growth inhibition of different freshwater phytoplankton species, which ranged over three orders of magnitude (Blanck et al. 1984). The close relationship between the concentration of arsenate that affects the structure and cell volume of phytoplankton communities and that which affects carbon dioxide fixation also implies that the ecologically important toxic effect of arsenate on phytoplankton is the inhibition of carbon dioxide fixation.

Induced Tolerance

The increased tolerance in carbon dioxide fixation of communities coming from the "high" corral was consistent with the PICT hypothesis (Blanck et al. 1988) which states that increased tolerance should be a consequence of selective suppression of sensitive organisms when a community is affected by a toxicant.

In marine periphyton communities, arsenate was shown to favour certain species (Blanck and Wängberg 1988a). Ideally the taxonomic composition of affected communities, presented in this paper, should have similarities that would distinguish them as a group from the communities not exposed to arsenate. Such a distinction was sought, using SIMCA, but none was found, probably because other environmental and seasonal events were more important than arsenic for determining the main structure of the communities (Fig. 7). A more fruitful strategy was to look at single taxa (Fig. 9). This implies that the increased tolerance for arsenate was induced by a selection of more tolerant taxa. Changes in species composition were not found by Planas and Lamarche (1983) even though they were adding much higher arsenic concentrations (30 μM), and phytoplankton cell volume decreased to less than 20% (estimated from their fig. 2). Their analysis was, however, limited to those species that represented over 10% of the total cell density which might explain the discrepancy.

Using the PICT hypothesis, it was possible to detect disturbances in microalgal communities in October (Table 3) which were not found by analysing their structure alone with the Czekanowski dissimilarity index (Fig. 6). We had earlier tested the PICT hypothesis on marine periphyton communities and arsenate with positive results (Blanck and Wängberg 1988b). As the toxicity of arsenate to microalgae is influenced

by the nutrient conditions (Wängberg and Blanck 1990) and the lake in this study differs markedly from the earlier tested marine environments, the generality of the PICT concept is supported. This is also the first test of the PICT hypothesis using phytoplankton communities.

One unexplained anomaly was observed. The phytoplankton community from the "medium" corral showed similar structural differences (Table 4) as the communities from the "high" corral, especially in August. For example, several taxa (Ochromonadaceae spp. No. 1 (3–4 μm), *Tabellaria fenestrata*, *Rhizosolenia longiseta*) that had extreme abundances in the "high" corral in October and in the affected communities during summer had even more extreme abundances in the "medium" corral in August. Phytoplankton from the "medium" corral, however, did not show any increased tolerance (Fig. 8).

There are three possible explanations for this anomaly. First, in August, when the structural similarities between the summer communities were strongest, the precision of the photosynthesis measurements was low (Table 2) and consequently small increases in tolerance may have been overlooked.

Second, the differences in abundance of single taxa that we used as an indicator of arsenate-affected communities may have been due to factors other than the arsenate stress, e.g. differences in abundance in the initial communities and for divergence in community structure as a result of the random walk phenomenon. Significant ($P > 0.1$, Mann–Whitney U-test) differences in abundance between the control and the "medium" or "high" corrals before the start of the arsenate addition were observed for four of the eight taxa having extreme abundances in the affected communities in Fig. 9. Only for *Gymnodinium ubberimum* in the "medium" corral was the difference in the same direction. For *Polytoma granuliferum*, *Cryptomonas* sp., and *Gymnodinium ubberimum* in the "high" corral the abundances were higher than in the control corral at the start of the experiment. Differences in the initial communities can thus not explain the recorded differences during summer. The positive correlation for several taxa in the tolerant communities and abundances in all affected communities during summer (Fig. 9), and lack of a negative correlation for any taxon, favours the interpretation that the changes in community structure are due to arsenate exposure and not to random divergence.

Third, differences in water quality (e.g. humus content) might have modified the availability of arsenate. We observed that in the October sampling the water in the "medium" corral was much clearer, but this was not confirmed by any measurements. Lower amounts of arsenate-binding substances in the "medium" corral would explain why the phytoplankton cell volume decreased more there than in the "high" corral during August–September (Fig. 4) despite the arsenic concentration being lower. The tolerance of algae would be underestimated when incubated in low humic water. There is a similarity between the dose–response curves for "medium" corral phytoplankton (Fig. 8b) and lake phytoplankton incubated with arsenate solutions in distilled water (Fig. 2), which supports this idea.

Arsenate Toxicity

It is expected that the majority of the arsenic is in the form of arsenate in aerobic waters at neutral pH (Woolson 1977). The natural background concentrations of arsenic in freshwaters are in the range 1–32 nM and arsenate is the most toxic arsenic species in nutrient-limited conditions (Blanck et al. 1989). Extrapolation of our toxicity data, the no-effect concentration being equal to or less than 43 nM, to these waters leads to the conclusion that already the natural background of arsenic may be a structuring factor for the microalgal communities in several freshwaters. A transformation to arsenic species other than arsenate, or decreased availability of arsenate due to binding to dissolved substances, might, however, reduce the toxicity of the arsenic. Few measurements have been made on arsenic speciation and its availability to algae in aerobic, neutral freshwaters. Brunskill et al. (1980) found few indications of reduction of arsenate in experimental enclosures during aerobic conditions, but during anaerobic conditions arsenate (As(V)) was rapidly reduced to arsenite (As(III)). Andreae (1978) reported low As(V) to As(III) ratios in some freshwaters. Although pH and oxygen conditions are important for arsenic speciation, no information was given for these sites.

A comparison with marine periphyton communities from the Swedish west coast (Blanck and Wängberg 1988a) shows that the freshwater communities studied in this paper were much more sensitive to arsenate. In both the long-term and short-term tests, freshwater communities were affected by about one tenth of that concentration needed to affect the marine communities at stable low-nutrient conditions. Although the total phosphorus to arsenic ratio (calculated from data in Blanck and Wängberg 1988a and Wängberg and Blanck 1990) in the sea of 42–57 (mol/mol) was slightly higher than the value recorded in this study, a more important difference between the marine and freshwater environments is the nutrient limitations. A higher sensitivity to arsenate was expected in phosphorus-limited lakes than in nitrogen-limited coastal waters. It is not known if the nutrient condition is the only reason for the difference, or if there also are basic qualitative differences in metabolism between marine and freshwater algae.

Acknowledgements

The hospitality of the staff at Grimsö Wildlife Research Station is gratefully acknowledged. Dr. Peter Blomqvist is especially thanked for counting phytoplankton and commenting constructively on the manuscript. Our work was financially supported by the National Swedish Environmental Protection Board (grant 5333071-5333076).

References

ANDREAE, M. O. 1978. Distribution and speciation of arsenic in natural waters and some marine algae. 25: 391–402.

AVRON, M., AND A. T. JAGENDORF. 1959. Evidence concerning the mechanism of adenosinetriphosphate formation by spinach chloroplasts. J. Biol. Chem. 234: 967–972.

BLANCK, H. 1985. A simple, community level, ecotoxicological test system using samples of periphyton. Hydrobiologia 124: 251–261.

BLANCK, H., K. HOLMGREN, L. LANDNER, H. NORIN, M. NOTINI, A. ROSEMARIN, AND S. SUNDELIN. 1989. Advanced hazard assessment of arsenic in the Swedish environment, p. 256–328. *In* L. Landner [ed.] Advanced hazard assessment in the aquatic environment. Springer Verlag, Berlin.

BLANCK, H., AND S.-Å. WÄNGBERG. 1988a. Validity of an ecotoxicological test system: short-term and long-term effects of arsenate on marine periphyton communities in laboratory systems. Can. J. Fish. Aquat. Sci. 45: 1807–1815.

1988b. Induced community tolerance in marine periphyton established under arsenate stress. Can. J. Fish. Aquat. Sci. 45: 1816–1819.

BLANCK, H., G. WALLIN, AND S.-Å. WÄNGBERG. 1984. Species dependent variation in algal sensitivity to chemical compounds. Ecotoxicol. Environ. Saf. 8: 339–351.

BLANCK, H., S.-Å. WÄNGBERG, AND S. MOLANDER. 1988. Pollution-induced community tolerance (PICT) — a new ecotoxicological tool, p. 219–230. *In* J. Cairns Jr. and J. A. Pratt [ed.] Functional testing of aquatic biota for estimating hazard of chemicals. ASTM STP 988, American Society for testing and materials, Philadelphia, PA.

BLOMQVIST, P., AND U. HEYMAN. 1989. Effects of arsenate additions to pelagic mesocosm on phytoplankton biomass and composition. Limnologica (Berlin) 20(2): 329–338.

BLOMQVIST, P., U. HEYMAN, AND R. GRUNDSTRÖM. 1981. The structure of the pelagic ecosystem in Lake Siggeforasjön. Scripta Limnologica Upsaliensia A:522, Uppsala, Sweden. 79 p.

BORG, H., K. HOLM, AND K. HOLMGREN. 1985. Determination of arsenic with automated hydride generation and flameless atomic absorption spectrometry. SNV PM 1985. National Swedish Environmental Protection Board, Box 1302, S-171 25 Solna, Sweden. 26 p. (In Swedish)

BRUNSKILL, G. J., B. W. GRAHAM, AND J. W. M. RUDD. 1980. Experimental studies on the effect of arsenic on microbial degradation of organic matter and algal growth. Can. J. Fish. Aquat. Sci. 37: 415–423.

CLARK, K. R., AND R. H. GREEN. 1988. Statistical design and analysis for a 'biological effects' study. Mar. Ecol. Prog. Ser. 46: 213–226.

GREIG-SMITH, P. 1983. Quantitative plant ecology. Studies in ecology. Vol. 9. 3rd ed. Blackwell Scientific Publications, Oxford. 359 p.

KOROLEFF, F. 1976. Determination of phosphorus, p. 117–126. *In* K. Grasshoff [ed.] Methods of seawater analysis. Verlag Chemie, Weinheim and New York.

LANDNER, L., H. BLANCK, U. HEYMAN, A. LUNDGREN, M. NOTINI, A. ROSEMARIN, AND B. SUNDELIN. 1989. Community testing, microcosm and mesocosm experiments: ecotoxicological tools with high ecological realism, p. 216–254. *In* L. Landner [ed.] Advanced hazard assessment in the aquatic environment. Springer Verlag, Berlin.

PLANAS, D., AND F. P. HEALEY. 1978. Effects of arsenate on growth and phosphorus metabolism in phytoplankton. J. Phycol. 14: 337–341.

PLANAS, D., AND A. LAMARCHE. 1983. Lack of effect of arsenic on phytoplankton communities in different nutrient conditions. Can. J. Fish. Aquat. Sci. 40: 156–161.

RAND, M. C., A. E. GREENBERG, J. T. TARAS, AND M. A. FRANSON [ED.]. 1976. Standard methods for the examination of water and wasterwater. 14th ed. American Public Health Association, Washington, DC.

SANDERS, J. G. 1979. Effects of arsenic speciation and phosphate concentration on arsenic inhibition of *Skeletonema costatum* (Bacillariophyceae). J. Phycol. 15: 424–428.

SCHINDLER, D. W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 6–25.

SIEGEL, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill International Book Company, Minneapolis, MN.

UTERMÖHL, H. 1958. Zur Vervollkomnung der quntitativen Phytoplanktonmethodik. Mitt. Int. Ver. Limnol. 9: 1–38.

WÄNGBERG, S.-Å., AND H. BLANCK. 1990. Arsenate tolerance in marine periphyton communities under different nutrient regimes. J. Exp. Mar. Biol. Ecol. 139: 119–134.

WOLD, S., C. ALBANO, W. J. DUNN III, U. EDLUND, K. ESBENSEN, P. GELADI, S. HELLBERG, E. JOHANSSON, W. LINDBERG, AND M. SJÖSTRÖM. 1983. Multivariate data analysis in chemistry, p. 17–96. *In* B. R. Kowalski [ed.] Proc. NATO Adv. Study Inst. on Chemometrics, Cosenza, Italy, Sept. 1983. Reidel Publ. Co., Dordrecht, Holland.

WOOLSON, E. A. 1977. Fate of arsenicals in different environmental substrates. Environ. Health Perspect. 19: 73–81.

Effect of Nile Tilapia (*Oreochromis niloticus*) on the Ecosystem of Aquaculture Ponds, and Its Significance to the Trophic Cascade Hypothesis[1]

James S. Diana, Daniel J. Dettweiler, and C. Kwei Lin[2]

School of Natural Resources and Center for Great Lakes and Aquatic Sciences, University of Michigan, Ann Arbor, MI, 48109-1115, USA

Diana, J. S., D. J. Dettweiler, and C. K. Lin. 1991. Effect of Nile tilapia (*Oreochromis niloticus*) on the ecosystem of aquaculture ponds, and its significance to the trophic cascade hypothesis. Can. J. Fish. Aquat. Sci. 48: 183–190.

The trophic cascade hypothesis holds that an increase in fish biomass causes a decline in food organisms of the fish, which cascades down to regulate lower trophic levels by altered consumptive demands. This hypothesis was evaluated by stocking Nile tilapia (*Oreochromis niloticus*) at densities of 0, 1, 2, or 3 fish/m^3 in triplicated 220-m^3 ponds near Bangkok, Thailand. All ponds were fertilized with 500 kg chicken manure·ha^{-1}·wk^{-1}. We measured phosphorus, nitrogen, chlorophyll *a*, primary productivity, zooplankton abundance, and fish yield in all ponds. Increased stocking density resulted in decreased adult fish growth but similar yields among the three density treatments. Regressions relating the abundance of each trophic level to the next higher trophic level were either positive or nonsignificant, which is contrary to trophic cascade predictions. Zooplankton became significantly more dense over time in fishless ponds than the other three treatments, but no other trophic level differed among treatments. These results indicate that presence of fish had a significant effect on zooplankton in ponds, but biomass of fish or abundance of zooplankton did not significantly affect other trophic levels.

Selon l'hypothèse des niveaux trophiques en cascade, une augmentation de la biomasse de poisson entraîne une baisse des proies qui vient régulariser les niveaux trophiques inférieurs en modifiant les besoins alimentaires. On a évalué cette hypothèse en ensemençant du tilapie (*Oreochromis niloticus*) à des densités de 0, 1, 2 ou 3 poissons/m^3 dans des étangs de 220 m^3 (essai triple) situés près de Bangkok, en Thaïlande. Tous les étangs ont été fertilisés avec du fumier de volaille à un taux de 500 kg·ha^{-1}·sem^{-1}. Les teneurs en phosphore, en azote et en chlorophylle *a*, la productivité primaire, l'abondance du zooplancton et le rendement en poisson ont été quantifiés dans tous les étangs. Une augmentation de la densité à l'ensemencement a entraîné une baisse du taux de croissance chez les poissons adultes, mais a donné des rendements semblables aux trois densités d'ensemencement. Les courbes de régression établissant un rapport entre l'abondance notée à un niveau trophique et le niveau trophique suivant étaient positives ou non significatives, en opposition avec les prévisions de l'hypothèse sur les niveaux trophiques en cascade. Le zooplancton est devenu nettement plus dense en fonction du temps dans les étangs libres de poisson par rapport aux trois autres étangs empoissonnés, mais aucun des autres niveaux trophiques n'a varié selon le traitement. Les résultats obtenus révèlent que la présence de poisson a une incidence significative sur le zooplancton dans les étangs, mais que la biomasse du poisson ou l'abondance du zooplancton n'a pas une incidence significative sur les autres niveaux trophiques.

Received February 13, 1990
Accepted August 29, 1990
(JA469)

Reçu le 13 février 1990
Accepté le 29 août 1990

The productivity of aquatic systems is of primary concern to aquaculturists and other aquatic biologists. The most widely accepted theory for food web productivity is that of resource limitation, or producer-controlled systems. This theory was formally delimited by Lindeman (1942) as the trophic dynamic viewpoint. It holds that productivity in aquatic ecosystems is limited by nutrient availability and its effect on primary production. Energy fixed by autotrophs is then competed for and consumed by heterotrophs, whose production is ultimately limited by energy available in autotrophic organisms. This approach has been termed a "bottom-up" approach (Carpenter et al. 1985; McQueen et al. 1986; Vanni 1987). This hypothesis has been broadly supported by data from a variety of systems (Schindler 1978; Mills and Schiavone 1982). Support of the hypothesis usually takes the form of establishing a significant positive correlation between primary production (or some correlate) and fish yield (Liang et al. 1981).

An alternative approach to understanding aquatic system productivity is the trophic cascade hypothesis (Carpenter et al. 1985). This hypothesis holds that food webs are also regulated by consumption from higher trophic levels. It is based on observations that predator populations, through their consumptive demands, may regulate the abundance of their prey. While numerous authors have demonstrated the effects of predators on their prey populations (Brooks and Dodson 1965; Galbraith 1967; Zaret 1972), the trophic cascade hypothesis predicts that these effects will lead to changes at lower trophic levels as well (Carpenter et al. 1985). A correlate of this hypothesis is that predators (usually fish), by changing the size distribution of

[1]Contribution 1049 of the CRSP Aquaculture Project and Contribution 530 of the Center for Great Lakes and Aquatic Sciences, University of Michigan.

[2]Present address: Agriculture and Food Engineering Division, Asian Institute of Technology, Bangkok, Thailand.

their prey (usually zooplankton), alter predation pressure on lower trophic levels and change productivity. Thus, the changes in size of zooplankton may be as or more important than changes in absolute zooplankton abundance. The trophic cascade has been termed "top-down" control of productivity (McQueen et al. 1986).

Tests of the trophic cascade have essentially followed three methods. The first is a comparison of enclosures in a lake, each of which is stocked with a different biomass of fish and then compared for trophic structure (McQueen et al. 1986; Mazumder et al. 1988). The second method of evaluation is to examine annual changes in trophic dynamics of a lake which has had a major perturbation (fish stocked or eradicated) (Scavia et al. 1986). The third method is to examine differences in food webs among a series of similar lakes which may differ in the composition or abundance of the fish community (Mills and Schiavone 1982). In all cases the tests compare strength and direction of regressions between consumer biomass (x-axis) and producer biomass (y-axis). Data may be collected weekly, monthly, or once per growing season. The frequency and nature of sampling has some influence on assessing the trophic cascade, as turnover rates at each trophic level are quite different, and therefore, one may wish to evaluate ultimate ("equilibrium") communities rather than the ecosystem immediately after a perturbation (Carpenter et al. 1985). The trophic cascade hypothesis would be supported if the regressions between adjacent trophic levels are negative. One might also expect the strength of these regressions (r^2) to decrease as one moved down the food web, as bottom-up effects may mask the effect of changes in fish biomass in trophic levels more remote from fish (McQueen et al. 1986).

Aquaculture ponds are excellent systems to test some aspects of the trophic cascade hypothesis. They are usually stocked with small animals at high density. These animals grow raipdly and produce a very large biomass. Density-dependent growth (and hence limited food resources) commonly occurs in ponds. Thus, fish-mediated effects are probably stronger in ponds than in natural systems with lower fish density. Ponds can be experimentally altered in a controlled manner, and therefore, one can test the hypothesis using the first method described. They also show large differences in fish biomass (due to growth) during a growing season, so they can be used for method 2 or 3. As control and management of productivity is important in aquaculture, it is of practical value to measure the potential impacts that resource limitation or the trophic cascade may have on fish yield. Ponds are notoriously variable, even when treated with the same inputs (Lannan et al. 1986; Diana et al. 1991). Thus, it is possible for some combination of these two productivity paradigms to aid in our understanding of this natural between-pond variation. As pointed out by Carpenter et al. (1985), the trophic cascade and resource limitation are likely complementary, as potential productivity is undoubtedly set by nutrient supply, while realized productivity may be altered by recycling rates and other top-down effects.

The purpose of this study is to determine if the trophic cascade hypothesis can be demonstrated with tilapia culture data, and whether this may help clarify variations in yield between ponds treated similarly. Twelve ponds fertilized at a constant rate were stocked with Nile tilapia (*Oreochromis niloticus*) at various densities. The trophic cascade was evaluated by comparing biomass or density (for zooplankton) between trophic levels using three data sets: (1) average data for each pond; (2) monthly data for each pond; and (3) average monthly data for each treatment.

Methods

Experiments were conducted at the Ayutthaya Freshwater Fisheries Station at Bang Sai, approximately 60 km northwest of Bangkok, Thailand (14°45′N, 100°32′E). Ponds used were 220 m^3 in volume when filled to a normal depth of 0.9 m. Three replicate ponds were randomly assigned to each of four treatments: densities of 0, 1, 2, or 3 fish/m^3. *Oreochromis niloticus* were stocked on 2 February 1987 at an average size of 32 g. The fish were manually sorted to sex prior to stocking, and only males were stocked. Manual sorting is not perfect, and on average, 5% of the fish stocked were females. Ponds were fertilized with chicken manure (assayed at 2.8–4.5–3.1 nitrogen–phosphorus–potassium) at a rate of 500 $kg \cdot ha^{-1} \cdot wk^{-1}$. This rate was determined to be good for fish production and primary production in earlier experiments (Diana et al. 1991). Experiments continued until 1 July 1987 (150 d), at which time the ponds were drained and all fish harvested.

All ponds were sampled monthly for physical and biological characteristics. Detailed sampling methods are outlined in CRSP (1989). Water was sampled by pooling three 90-cm column samples of water per pond, taken at 10:00 a.m. Ammonia, nitrate, nitrite, and total phosphorus were measured using standard methods on filtered water (APHA 1980). The phenate procedure was used to determine ammonia, while the phenodisulfonic procedure was used for nitrate and nitrite determination. Total inorganic nitrogen was considered the sum of the three inorganic nitrogen values. Persulfate was used for digestion of total phosphorus samples prior to analysis (USEPA 1979). Chlorophyll *a* was measured monthly according to methods detailed by Wetzel and Likens (1979), using the same water sample collected for nutrient analysis. Chlorophyll *a* concentration (milligrams per cubic metre) was considered a reasonable approximation of phytoplankton biomass.

Zooplankton composition was estimated monthly from three 90-cm water column samples per pond. Zooplankton was counted (number per litre) and sorted into rough categories of rotifers, copepods, copepod nauplii, protozoans, cladocerans, and ostracods. Weights of zooplankton were not taken, and numbers (mainly composed of rotifers) were considered an adequate representation of zooplankton biomass. Since numbers would reflect biomass only if similar-sized zooplankton were counted, we further divided zooplankton counts into large-bodied zooplankton (cladocerans and copeods) and small-bodied zooplankton (rotifers, protozoans, and nauplii).

Fish stocked initially in each pond were counted, sexed, and batch weighed. Adult fish weight was estimated monthly in each pond by weighing fish from cast-net samples representing 10% of the initial number stocked. At termination of experiments, all fish were removed from ponds, weighed, and counted. Adult fish numbers were estimated monthly by linear interpolation from the initial and final fish numbers. This was reasonable, since survival exceeded 88% in all ponds and did not differ among treatments. Monthly mean biomass of adult tilapia was calculated from mean individual weight multiplied by monthly number of fish. Offspring were produced in all ponds and were collected by seine, weighed (to determine offspring biomass), and removed at monthly intervals. Total biomass of tilapia was calculated by adding adult and offspring biomass, either monthly or over the entire grow-out.

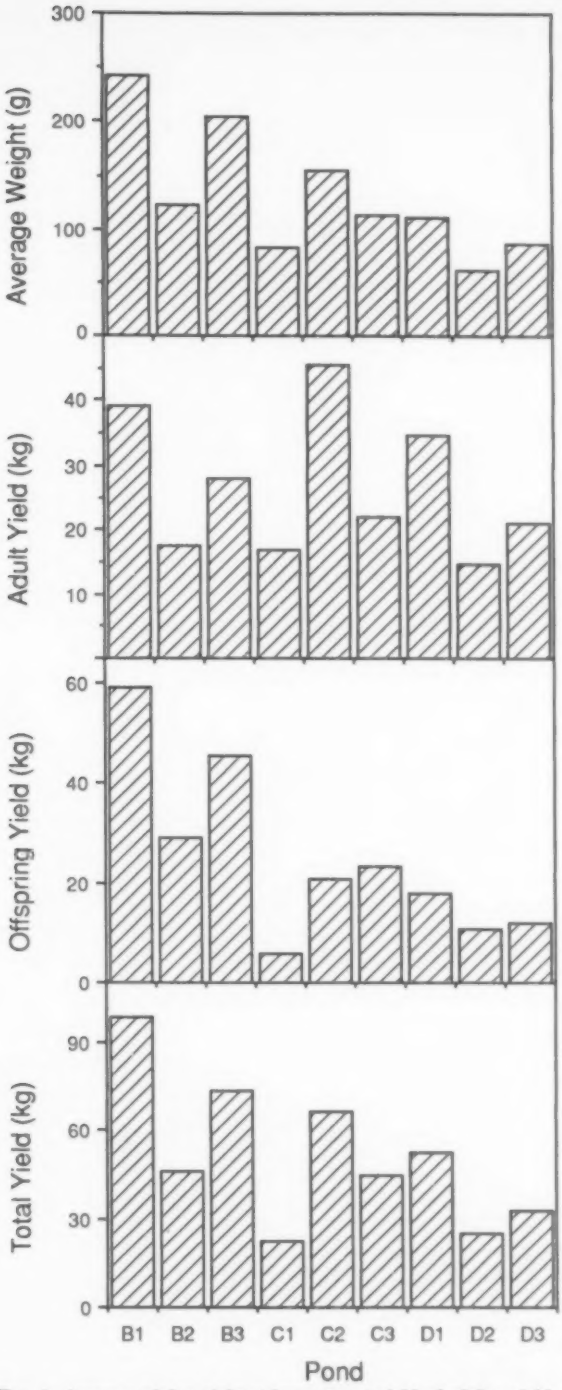


FIG. 1. Average adult weight at harvest, net yield of adults, yield of offspring, and total yield achieved in the nine experimental ponds with fish. B, C, and D are treatments with 1, 2, or 3 fish/m^3.

Regression and ANOVA analyses were done using the MIDAS program (Fox and Guire 1976). Data were tested for homogeneity of variances using Box's modification of Bartlett's test. Data which had heterogeneous variances were $\log_{10}$ transformed, which usually produced homogeneous variances.

TABLE 1. Effects of fish density on growth and yield of *Oreochromis niloticus* in ponds. p = results of ANOVA test between treatments.

Variable	Density	Value (mean ± 1 SE)	p
Average adult weight (g)	1	190 ± 35.5	0.06
	2	117 ± 20.9	
	3	86 ± 13.8	
Net adult yield (kg)	1	28.2 ± 6.3	0.85
	2	20.8 ± 8.8	
	3	23.4 ± 5.9	
Offspring yield (kg)	1	44.5 ± 8.8	0.02
	2	16.6 ± 5.5	
	3	9.4 ± 4.7	
Total net yield (kg)	1	72.7 ± 15.0	0.17
	2	44.7 ± 12.7	
	3	36.8 ± 8.0	

Time-related differences among treatments in physical or biological data were tested using a repeated measures test and the program Systat.

Results

Increased stocking density of tilapia resulted in decreased adult growth, but similar yield. Density-dependent growth was evident, but much variation occurred between average size of fish from ponds of a similar treatment (Fig. 1). However, there was a marginally significant effect of stocking density on individual weight at harvest (ANOVA, $p < 0.10$) (Table 1). This is evidence for resource limitation and competition affecting fish yield. Net adult yield was not significantly different among treatments, indicating that all ponds probably reached carrying capacity by the end of the experiment. Production of offspring was significantly different among treatments, with low-density treatments producing the most offspring. Differences in offspring yield were not large enough to cause a significant difference in total yield among treatments, mainly because of high variability between ponds within a treatment for both adult and offspring yield (Fig. 1).

Regressions based on either overall average data or monthly sampling did not support the trophic cascade hypothesis (Table 2; Fig. 2). Average data for an entire grow-out usually showed the highest correlation coefficients, but the slopes of all five significant regressions were positive and nine regressions were not significant. Nearly all of the regressions for monthly samples were significant: only 2 of 14 had negative slopes, and these were both nonsignificant (Table 2). The positive slopes on overall data again indicate support for resource limitation, although there was large variance among ponds (low r^2). Examining zooplankton abundance by size class showed that abundance of small-bodied zooplankton showed similar trends to total zooplankton, while large-bodied zooplankton had no significant relationship to fish biomass ($p = 0.61$). Curiously, for monthly data sets the overall zooplankton abundance and the abundance of small-bodied zooplankton had similar relationships to fish biomass, while for overall average data, the abundance of small-bodied zooplankton was much more strongly correlated with fish biomass than was abundance of

TABLE 2. Summary of regressions to test top-down relationships in tilapia culture ponds. All = all ponds; fish = only ponds with fish.

Comparison	Slope	n	r^2	p
Monthly data				
Total fish – zooplankton	0.54	45	0.19	0.001
Total fish – small zooplankton	0.33	45	0.20	0.002
Adult fish – zooplankton	0.55	45	0.18	0.004
Adult fish – small zooplankton	0.58	45	0.20	0.002
Total fish – chlorophyll *a*	0.50	45	0.17	0.001
Adult fish – chlorophyll *a*	0.49	45	0.15	0.008
Zooplankton – chlorophyll *a*				
All	0.43	60	0.29	0.001
Fish	0.50	45	0.26	0.001
Small zooplankton – chlorophyll *a*				
All	0.42	60	0.28	0.001
Fish	0.49	45	0.25	0.001
Chlorophyll *a* – nitrogen				
All	−0.09	60	0.02	0.232
Fish	−0.07	45	0.02	0.406
Chlorophyll *a* – phosphorus				
All	0.20	60	0.05	0.081
Fish	0.14	45	0.02	0.332
Average data				
Total fish – zooplankton	0.23	9	0.11	0.372
Total fish – small zooplankton	1.14	9	0.56	0.020
Adult fish – zooplankton	0.47	9	0.32	0.114
Adult fish – small zooplankton	1.59	9	0.72	0.003
Total fish – chlorophyll *a*	0.77	9	0.84	0.001
Adult fish – chlorophyll *a*	0.92	9	0.81	0.001
Zooplankton – chlorophyll *a*				
All	0.23	12	0.16	0.196
Fish	0.61	9	0.25	0.170
Small zooplankton – chlorophyll *a*				
All	0.20	12	0.27	0.083
Fish	0.43	9	0.62	0.011
Chlorophyll *a* – nitrogen				
All	−0.28	12	0.20	0.145
Fish	−0.01	9	0.00	0.922
Chlorophyll *a* – phosphorus				
All	−0.17	12	0.05	0.485
Fish	−0.04	9	0.00	0.909

total zooplankton (Table 2). Once again, these regressions had positive slopes, supporting bottom-up effects.

A comparison of the differences among treatments over time (Fig. 3) indicated that only zooplankton populations differed significantly with treatment (repeated measures test, $p < 0.05$). Indeed, the only significant difference was that zooplankton in fishless ponds were much more abundant than in ponds with fish. There were no significant differences among other density treatments in zooplankton numbers. This was true for total zooplankton abundance (Fig. 3) or for small-bodied zooplankton abundance (Fig. 4). However, there was no effect of density treatment on abundance of large-bodied zooplankton. Chlorophyll *a*, total phosphorus, and total inorganic nitrogen also had no significant treatment effects (log-transformed data due to heterogeneous variances). The only trophic level which showed significant trends over time was zooplankton, which increased significantly over time in fishless ponds.

There was considerable variation in average abundance of large-bodied zooplankton, small-bodied zooplankton, and chlorophyll *a* concentration between replicates of a density treatment (Fig. 4), but no significant treatment effects for any characteristic except small-bodied zooplankton abundance and fish density (repeated measures test, $p < 0.05$). Once again, the only significant difference occurred between a density of 0 or 1 fish/m^3, indicating that presence of fish reduced the abundance of small-bodied zooplankters.

Analyses so far included all data from pond flooding to harvest. As individual ponds were filled at different times, data from the first few months may not represent stable or similar conditions. Most changes in abundance at each trophic level occurred over the first 2 mo, and the ponds had relatively stable conditions over the last 3 mo (Fig. 3). Attempts to analyze the trophic cascade under these later months did not improve the fit of the regressions, whether data included were the variables for the last 3 mo, the means of these, or the last data point collected. Therefore, attempts to account for stable conditions did not support the trophic cascade hypothesis any better than overall data.

In the monthly data, the regression for zooplankton density-chlorophyll *a* concentration was significant. The residuals of that relationship were regressed against the log of total fish biomass, offspring biomass, and adult fish biomass (Table 3) in an attempt to look at biomass of higher trophic levels as the factor causing variation in the relationship between chlorophyll and zooplankton. Two of these regressions were nonsignificant, and the only significant one (offspring biomass against the residuals) had an r^2 of only 0.23. For overall data, the regression between chlorophyll *a* and abundance of small-bodied zooplankton was significant for ponds with fish (Table 2). Residuals of this regression were more strongly correlated with adult and total fish biomass (Table 3). Therefore, variances in the small-bodied zooplankton – chlorophyll *a* regressions (which showed bottom-up effects) were strongly correlated with variations in fish biomass (top-down).

Discussion

The ponds used in these experiments clearly demonstrated that resource limitation occurred. Growth rate of adults was inversely proportional to density stocked, indicating strong density-dependent growth. This density dependence was so strong that yield was similar for each treatment. Density stocked also strongly affected reproduction, resulting in lower offspring

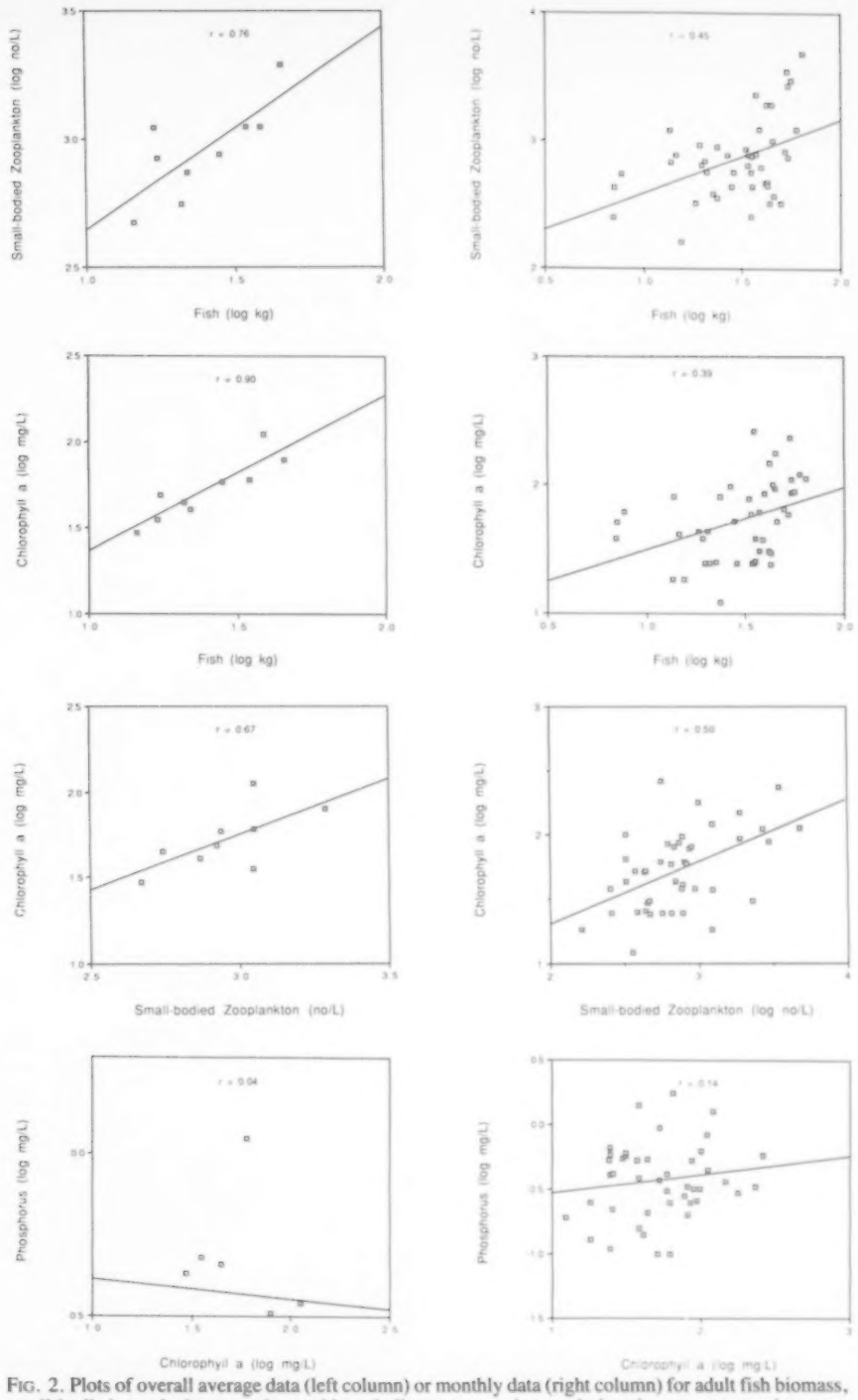


FIG. 2. Plots of overall average data (left column) or monthly data (right column) for adult fish biomass, small-bodied zooplankton numbers, chlorophyll *a* concentration, and phosphorus concentration compared by regression.

production in high-density ponds. This resulted in large but statistically insignificant differences in total fish yield, due mainly to variable production of offspring and possibly competition between offspring and adults in ponds of a similar treatment. All of these results indicate that resources were apparently limiting in these fertilized fish ponds, and that carrying capacity for adults was reached at a density of 1 fish/m^3. Density dependence in growth and survival has commonly been

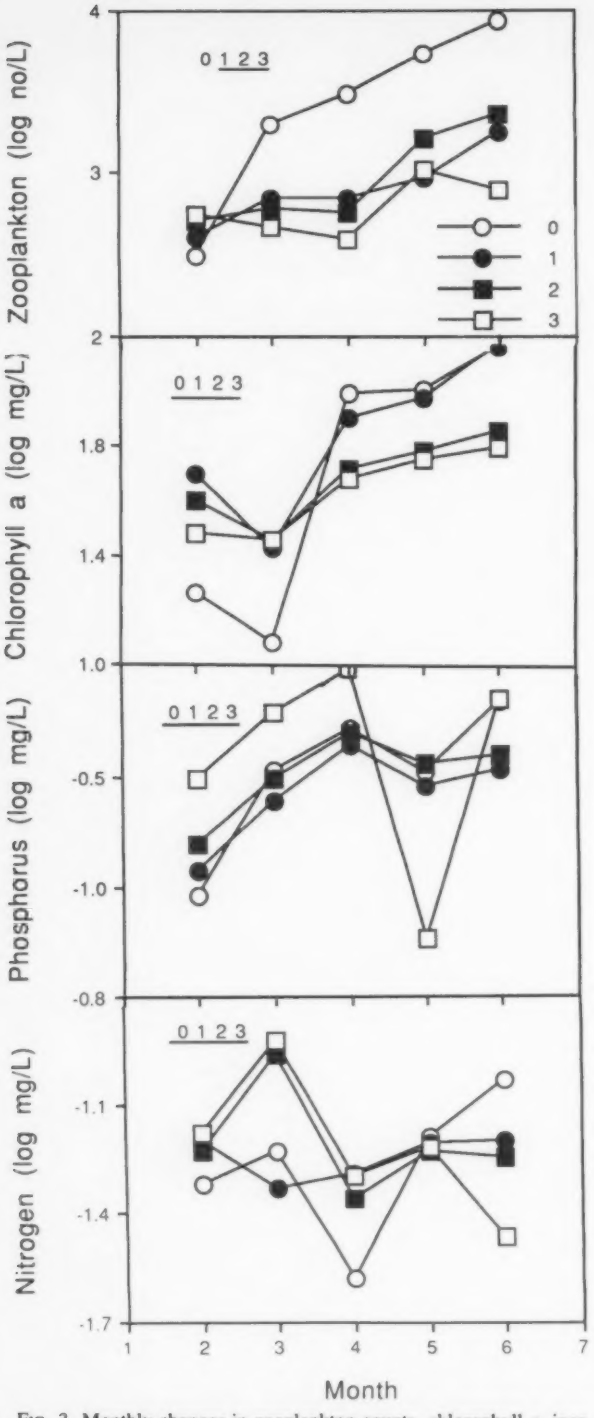


FIG. 3. Monthly changes in zooplankton counts, chlorophyll *a*, inorganic phosphorus, and nitrogen concentrations in ponds of each treatment (0, 1, 2, or 3 fish/m^3). Groups in the upper left corner of each box are not significantly different if they are underlined at the same level (repeated measures test, $p < 0.05$).

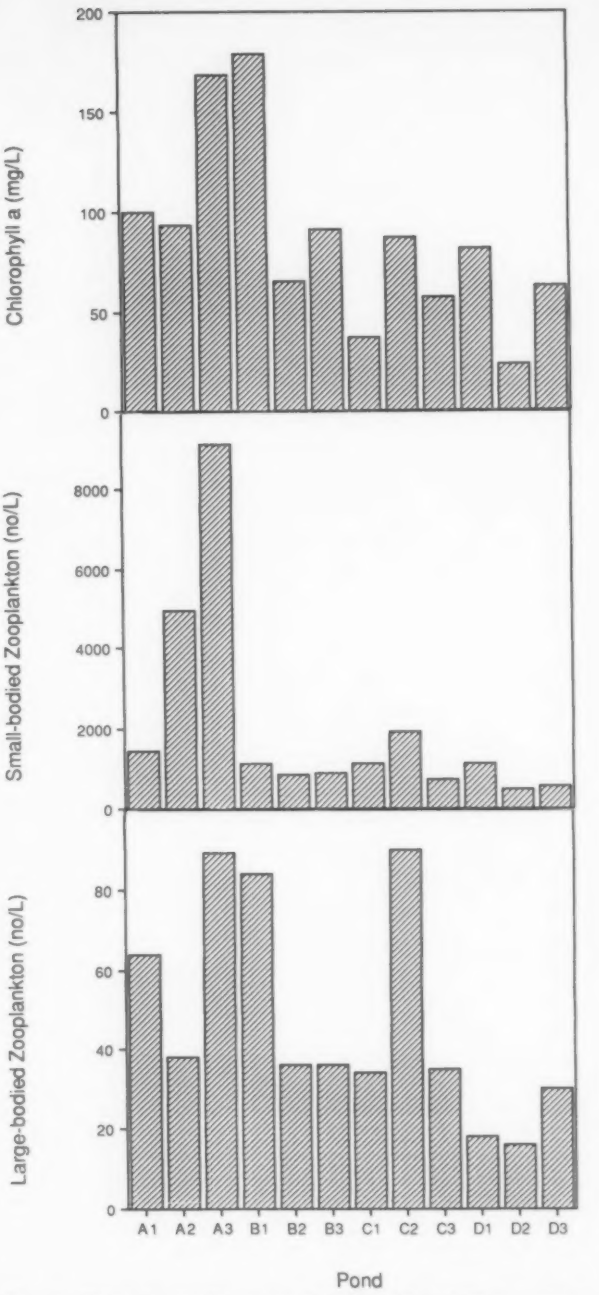


FIG. 4. Plots of overall average data for chlorophyll *a* content, small-bodied zooplankton, and large-bodied zooplankton in the 12 experimental ponds. A, B, C, and D are treatments with 0, 1, 2, or 3 fish/m^3.

found in fish ponds, indicating that resource limitation is important (Hepher 1978). This has led to the development of fertilization and supplemental feeding to increase food resources and yield.

In spite of large differences in standing crop and fish density among ponds, we were unable to show much evidence to support the trophic cascade hypothesis. Correlations between

TABLE 3. Regression statistics for the residuals of the chlorophyll – small-bodied zooplankton relationship against the logs of adult fish biomass, total fish biomass, and offspring biomass for monthly and average data.

Regression variables	Slope	n	r^2	p
	Monthly data			
Adult fish biomass residuals	0.250	45	0.05	0.132
Total fish biomass residuals	0.265	45	0.06	0.095
Offspring biomass residuals	0.50	45	0.27	0.001
	Average data			
Adult fish biomass residuals	0.612	9	0.63	0.010
Total fish biomass residuals	0.545	9	0.75	0.002
Offspring biomass residuals	0.135	9	0.23	0.190

trophic levels were always positive, indicating that resource limitation may control production, but not indicating any effect of fish on zooplankton or phytoplankton. Only comparing the residuals of regressions for average data between adjacent trophic levels with the higher trophic level biomass gave much indication of the trophic cascade effects. Fish apparently had little effect on the ecosystems that developed in the experimental ponds. Indeed, the only significant effect was that total zooplankton density and small-bodied zooplankton density were much higher in fishless ponds than in ponds with fish (ANOVA, Fig. 3 and 4). Although one might regard this as an indication that the main effects occurred between a density of 0 and 1 fish/m^3, there were no differences in phytoplankton or nutrient concentrations between these density treatments. Thus, the predators in this system (fish) did inhibit the zooplankton population, but this inhibition did not cascade down to other trophic levels. The level of inhibition was also minimal, as it was exhibited only when fishless ponds were compared with those with fish, and was not correlated with fish biomass or density. Fish had little consistent or predictable effect on the lower level food web dynamics of these fertilized fish ponds. Other studies have also failed to support the trophic cascade hypothesis in different systems (Scavia et al. 1986; McQueen et al. 1986; Vanni 1987). Effects of predators on their prey populations were also found in those studies (as in the present study), but the predator–prey interaction did not "cascade" to other trophic levels. Indeed, Vanni (1987) found that food availability was much more important in affecting zooplankton densities than was fish predation, which is consistent with our results.

The trophic cascade hypothesis predicts a rather simple ecological system where effects on one trophic level cascade directly (Carpenter et al. 1985). However, a difficulty in this analysis is that many fish are omnivorous and may consume organisms from several trophic levels. This is certainly true for tilapia (Balarin 1979; Bowen 1982). Small tilapia (less than 35 g) in our ponds eat mainly larger zooplankton, but then exhibit a dramatic shift to filter feeding (C. K. Lin, unpublished data). When filter feeding, they consume mainly phytoplankton and small zooplankton (rotifers), while large zooplankton can apparently avoid their filtering mechanism (C. K. Lin, unpublished data). Thus, different size classes of tilapia also utilize different food and, therefore, have variable effects on the prey community. Of course, variable food habits are not exclusive to tilapia, as many fish may have ontogenetic shifts in food preference or feed omnivorously at some age (Keast 1985). Fish which consume food from more than one trophic level obviously present difficulties to the trophic cascade hypothesis, as consumption effects of one organism variably affect the other trophic levels (Vanni 1987).

The present analyses have several problems which affect interpretation of the results, as our measurements were not directly comparable with other works on the trophic cascade. We used chlorophyll *a* as an indicator of phytoplankton biomass. While this is an indirect measure, it has commonly been used in similar analyses (McQueen et al. 1986). We also used zooplankton counts as indicators of biomass. To overcome difficulties related to size structure of zooplankton, we also separated zooplankton into large- or small-bodied groups. Zooplankton counts were dominated by rotifers. Analyses of the trophic cascade have often examined relationships between large zooplankton such as *Daphnia* and the phytoplankton population (McQueen et al. 1986; Scavia et al. 1986; Vanni 1987). In this study, zooplankton changes only occurred between 0 and 1 fish/m^3, and only for small-bodied zooplankton. Possibly, the change in abundance of large-bodied zooplankton is most important to the function of a trophic cascade, rather than changes in overall plankton abundance as in this study. If this is true, then the trophic cascade hypothesis can only be applied to interactions between predators and large-bodied zooplankton.

McQueen et al. (1986) suggested that top-down effects diminish at the base of the food chain, particularly in eutrophic systems. The present aquaculture ponds were much more productive than eutrophic lakes, with gross primary production often exceeding 5 g $C \cdot m^{-3} \cdot d^{-1}$ and chlorophyll *a* concentrations exceeding 10 mg/L (Diana et al. 1991). However, in spite of high nutrient loadings and high productivity, food resources became limiting in these ponds and fish growth declined with increased density. As fish prey abundance was apparently insufficient for continued good growth, tilapia did affect abundance of their prey (small zooplankton) significantly, but this did not cascade to lower trophic levels. This same result was seen in studies on oligotrophic (Scavia et al. 1986) to mesotrophic lakes (McQueen et al. 1986; Vanni 1987; Mazumder et al. 1988), so there really is no evidence that highly eutrophic systems respond any differently than less productive ones.

Ponds are inherently variable ecosystems. Management of ponds is largely an attempt to control the chemical and biological processes in ponds, and often results in variable outcomes (Lannan et al. 1986). In the present study, there was large variation in fish growth and the food web among ponds of the same treatment (Fig. 1 and 4). While there were some seasonal trends in chemical and biological characteristics of the ponds, the fertilization regime used in this study did result in relatively consistent phytoplankton and zooplankton populations over time (Fig. 3), at least in comparison with temperate ecosystems which show large seasonal changes in plankton populations (Barica 1975; Wetzel 1975). In fact, since the fish biomass appeared to be at carrying capacity by the end of the experiment, and pond conditions had stabilized by then, the ponds appeared to have reached a stable ecosystem by late in the experiment. Predictions of yields would be inconsistent for

these ponds, as there are large variations within treatments (Fig. 1). Regressions between various trophic levels and fish biomass (yield) are equally variable (Table 2). Determining the reasons for this variation is an important goal for future aquaculture research, and is necessary to allow better predictions of fish yield under most aquaculture systems.

Acknowledgements

This project was conducted with the cooperation of the National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. T. Bhukaswan, V. Sirsuwanatach, and S. Hiranyawat aided in logistics and data evaluation. The study was conducted at the Ayutthaya Freshwater Fisheries Station, which is directed by S. Ukatawewat. Data collection and laboratory analyses were done by V. Tansakul, S. Auworatham, W. Muthwana, and assistants. B. Diana aided in data analysis and text preparation. This research is a component of the Pond Dynamics/Aquaculture CRSP supported by the Agency for International Development Grant No. DAN-4023-G-SS-2074-00 and by contributions from participating institutions.

References

American Public Health Association (APHA). 1980. Standard methods for the examination of water and wastewater. 15th Ed. APHA, Washington, DC. 1134 p.

Balarin, J. D. 1979. Tilapia — A guide to their biology and culture in Africa. Institute of Aquaculture, University of Stirling, Stirling, Scotland. 174 p.

Barica, J. 1975. Collapses of algal blooms in prairie pothole lakes: their mechanism and ecological impact. Verh. Int. Ver. Limnol. 19: 606–615.

Bowen, S. H. 1982. Feeding, digestion, and growth-qualitative considerations, p. 141–156. *In* R. S. V. Pullin and R. H. Lowe-McConnell [ed.] The biology and culture of tilapias. ICLARM Conference Proceedings 7, International Center for Living Aquatic Resources Management, Manila, Philippines.

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size and the composition of plankton. Science (Wash., DC) 150: 28–35.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience 35: 634–639.

Collaborative Research Support Program (CRSP). 1989. CRSP work plan: fourth experimental cycle. Office of International Research and Development, Oregon State University, Corvallis, OR. 34 p.

Diana, J. S., C. K. Lin, and P. J. Schneeberger. 1991. Relationships among nutrient inputs, water nutrient concentrations, primary production, and yield of *Oreochromis niloticus* in ponds. Aquaculture 92. (In press)

Fox, D. J., and K. E. Guire. 1976. Documentation for MIDAS. Statistical Research Laboratory, The University of Michigan, Ann Arbor, MI. 203 p.

Galbraith, M. G. 1967. Size selective predation of *Daphnia* by rainbow trout and yellow perch. Trans. Am. Fish. Soc. 96: 1–10.

Hepher, B. 1978. Ecological aspects of warm-water fishpond management, p. 447–468. *In* S. D. Gerking [ed.] Ecology of freshwater fish production. John Wiley and Sons, New York, NY.

Keast, A. 1985. Planktivory in a littoral-dwelling lake fish association: prey selection and seasonality. Can. J. Fish. Aquat. Sci. 42: 1114–1126.

Lannan, J. E., R. O. Smitherman, and G. Tchobanoglous. 1986. Principles and practices of pond aquaculture. Oregon State University Press, Corvallis, OR. 240 p.

Liang, Y., J. M. Melack, and J. Wang. 1981. Primary production and fish yields in Chinese ponds and lakes. Trans. Am. Fish. Soc. 110: 346–350.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23: 399–417.

Mazumder, A., D. J. McQueen, W. D. Taylor, and D. R. S. Lean. 1988. Effects of fertilization and planktivorous fish (yellow perch) predation on size distribution of particulate phosphorus: large enclosure experiments. Limnol. Oceanogr. 33: 421–430.

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1571–1581.

Mills, E. L., and A. Schiavone. 1982. Evaluation of fish communities through assessment of zooplankton populations and measures of lake productivity. N. Am. J. Fish. Manage. 2: 14–27.

Scavia, D., G. L. Fahnenstiel, M. S. Evans, D. J. Jude, and J. T. Lehman. 1986. Influence of salmonid predation and weather on long-term water quality trends in Lake Michigan. Can. J. Fish. Aquat. Sci. 43: 435–443.

Schindler, D. W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol. Oceanogr. 23: 478–486.

U.S. Environmental Protection Agency (USEPA). 1979. Methods for chemical analysis of water and wastes. Office of Research and Development. EPA-600/4-79-020. USEPA, Cincinnati, OH.

Vanni, M. J. 1987. Effects of food availability and fish predation on a zooplankton community. Ecol. Monogr. 57: 61–88.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Philadelphia, PA. 743 p.

Wetzel, R. G., and G. E. Likens. 1979. Limnological analyses. W. B. Saunders Co., Philadelphia, PA. 357 p.

Zaret, T. M. 1972. Predator–prey interaction in a tropical lacustrine ecosystem. Ecology 53: 248–257.

Decomposition of Rainbow Trout (*Oncorhynchus mykiss*) Carcasses in a Forest Stream Ecosystem Inhabited only by Nonanadromous Fish Populations

G. Wayne Minshall and Evelyn Hitchcock

Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, USA

and James R. Barnes

Department of Zoology, Brigham Young University, Provo, UT 84602, USA

Minshall, G. W., E. Hitchcock, and J. R. Barnes. 1991. Decomposition of rainbow trout (*Oncorhynchus mykiss*) carcasses in a forest stream ecosystem inhabited only by nonanadromous fish populations. Can. J. Fish. Aquat. Sci. 48: 191–195.

The overall dynamics of rainbow trout (*Oncorhynchus mykiss*) carcass decomposition in a woodland stream ecosystem was examined in two experiments conducted in the West Fork of Mink Creek, Idaho: one during winter–spring (mean water temperature 4.2°C) and one during summer (mean water temperature 8.6°C). Relative weight loss (%AFDW) from fish during both periods was essentially constant. In spring, mean daily loss per day was 1.5%. Although this rate is comparable with the decay of high-quality ("fast") leaves, it took much longer than expected (>120 d) for the even higher quality fish protein. In summer, decay was more rapid (4.9%/d) and was completed in less than half the time (~50 d). Most decay appeared to progress from inside the carcasses outward to the skin. The skin remained intact throughout most of both experiments. Nutrients leached from the fish appeared to be utilized rapidly by microbes associated with the carcass, since no stimulation of algal growth occurred immediately downstream of the carcasses. This suggests extremely tight nutrient spirals and high nutrient retention in Mink Creek and other comparable Rocky Mountain headwater streams.

On a étudié la dynamique globale de la décomposition de carcasses de truite arc-en-ciel (*Oncorhynchus mykiss*) dans l'écosystème d'un ruisseau sylvestre par l'entremise de deux expériences menées dans la branche ouest du ruisseau Mink, dans l'État de l'Idaho : une des expériences a été réalisée au cours de l'hiver et du printemps quand la température moyenne de l'eau atteignait 4,2°C et l'autre pendant l'été quand la température moyenne de l'eau s'élevait à 8,6°C. La perte relative de poids (% AFDW) pendant ces deux périodes est demeurée relativement constante. Au printemps, la perte moyenne de poids par jour s'élevait à 1,5 %. Même si ce taux est comparable à la décomposition de feuilles de haute qualité (décomposition rapide), la décomposition des protéines de poisson de qualité supérieure a été plus lente que prévue (>120 jours). À l'été, la décomposition a été plus rapide (4,9 %/jour) et a pris moins de la moitié du temps (~50 jours). La plus grande partie de la décomposition semble s'effectuer de l'intérieur de la carcasse vers la peau. Celle-ci est demeurée intacte pendant presque toute la durée des deux expériences. Les bio-éléments libérés des tissus en décomposition semblent être utilisés rapidement par les microbes peuplant les carcasses étant donné qu'aucune stimulation de la croissance des algues n'a été notée immédiatement en aval des carcasses. Ceci porte à croire à la présence de spirales de bio-éléments très hermétiques et à un taux de rétention élevé des bio-éléments dans le ruisseau Mink et d'autres cours d'eaux d'amont comparables des montagnes Rocheuses.

Received April 2, 1990
Accepted August 27, 1990
(JA535)

Reçu le 2 avril 1990
Accepté le 27 août 1990

Although a number of investigations have been conducted concerning the decay of terrestrial plant leaves and woody debris in small woodland streams, comparable study of decomposition of animal matter has lagged far behind. A tacit assumption by most stream ecologists seems to be that animals are consumed directly by other animals and few, if any, die from injury, disease, or old age and undergo microbial decay. An exception is those who have encountered and/or dealt with mass movements of relatively large fish. Several of these investigators have noted the potential for decaying anadromous salmonid carcasses (*Oncorhynchus*) to stimulate growth of suspended and attached algae in spawning streams and lakes (Juday et al. 1932; Narver 1967; Mathisen 1972; Richey et al. 1975). A few researchers also have speculated that migratory fishes may be important in resupplying the nursery area with nutrients (Donaldson 1967; Hall 1972; Mathisen 1972), and this idea is supported by the work of Kline et al. (1990). The latter group has demonstrated the uptake of marine-derived carbon and nitrogen from anadromous salmonids by algae, macroinvertebrates, and resident fish. Apart from these investigations, virtually nothing is known of the fate, role, and temporal sequence of decomposition of dead fish or other animals in streams; we know of no study which has addressed these aspects in streams from which anadromous fish are absent.

This study was designed as an initial exploration of the dynamics of fish carcass decomposition in woodland stream ecosystems lacking anadromous fish. We describe changes in the gross morphology of carcasses, the rate of decomposition,

and the fate of the byproducts of decay under cool (winter–spring) and warm (summer) conditions.

Methods

Two experiments were conducted in the West Fork of Mink Creek, Bannock County, Idaho, USA (112°23′W, 42°48′N): one during winter–spring (7 February – 6 June 1987, mean temperature 4.2°C) and one during summer (17 June – 3 August 1987, mean temperature 8.6°C) in order to determine, at frequent intervals, gross changes associated with the decomposition of fish carcasses. Water temperature was measured continuously throughout both studies by means of a Peabody–Ryan submersible thermograph. Maximum and minimum values (winter–spring 0.5–11°C; summer 6.5–10.8°C, respectively) were read directly off the chart recording: daily means were estimated from hourly values. Degree-days were calculated by multiplying the mean values by the number of days (118 and 48, respectively) in the experiment. Rainbow trout (*Oncorhynchus mykiss*) (formerly *Salmo gairdneri*) from the Idaho Fish and Game American Falls Hatchery were placed on ice, transported to Idaho State University, and prepared for placement in Mink Creek. Freshly killed fish were weighed after excess surface water was removed by blotting. Half of the fish were placed individually in numbered 6-mm-mesh aquanet envelopes, and the other half were fitted with nylon cord tethers inserted around the jaw (winter–spring) or through the mouth and opercular opening (summer). Mean weight of fish was 102.97 g (N = 24, SD = 30.71) in spring and 145.63 g (N = 39, SD = 17.56) in summer; mean length was 12.78 cm (SD = 2.18) and 18.07 cm (SD = 3.81), respectively. Fish were frozen following weighing and held in that condition (<1 d) prior to transport to the study site. Sets of one tethered fish, one envelope-encased fish, and an empty envelope (arranged in alternating fashion among sets) were fastened with spikes in transects across the width of the stream at intervals of 1 m or more between transects. The empty envelopes served as controls for determining if there was a change in benthic macroinvertebrate abundance associated with decaying fish in envelopes. However, no differences in abundances or kinds were detected from gross examination of sorted samples and so no further analysis of these aspects was conducted.

At approximately 7-d intervals in spring and 4-d intervals in summer, three sets were brought into the laboratory for analysis. The fish were examined for condition, excess surface water blotted off, weighed, and then dried at 60°C for at least 3 d to obtain a whole final dry weight. The gross appearance of each fish was determined immediately upon retrieval from the stream in terms of fungal growth, internal condition, and external condition. Observations of fungal growth included measurement of the length of the fungus and the percent coverage of the fish surface. External observations included length and condition of fins, overall conformation, condition of head and eyes, and intactness of skin. Internal observations included texture of muscle, condition of organs, and presence of insects. For the winter–spring experiment, a representative cross-sectional slice was removed from the middle of the fish, weighed, and ashed (440°C for approximately 2 h). This information was used to determine total ash-free dry weight (AFDW) and percent organic matter (%OM) of fish after decomposition. For the summer experiment the weighing and ashing procedures were repeated, but since the state of decomposition generally was more rapid, the entire fish was analyzed. The mean percent dry weight and %OM of seven freshly killed fish were 24.49 and 84.88%, respectively. Dry weight and organic (ash-free) weight losses due to decomposition were determined from the initial and final weights for each collection date. A loss rate coefficient, k, was estimated by a least squares fit of the AFDW data to an exponential function (Petersen and Cummins 1974). At any period of time, t, the loss rate coefficient, k, is

$$\ln (\%\ \text{remaining}/100)/t = -k.$$

Periphyton standing crops on unglazed tiles (15.1 ± 0.1 × 7.5 ± 0.1 × 1.2 ± 1.2 ± 0.1 cm; N = 6) were used to assess possible release and uptake of nutrients from the fish carcasses. Tiles were placed immediately upstream, below, and downstream of the envelopes holding the decaying fish at the outset of each experiment. In winter–spring, the envelopes were placed directly over the "below" tiles, but this caused undue shading and so the protocol was changed. In summer the "below" tiles were located immediately downstream of the envelopes. Also in winter–spring, the tiles were placed loose on the stream bottom but a number were moved and/or turned over by the current. So, in summer, the tiles were fastened to 20-cm-wide by 100-cm-long strips of 6-mm-mesh plastic netting that were held in the stream with spikes. This arrangment also assured a fixed positioning of the tiles and the fish. The envelope of fish was placed in the center of each strip and one tile was fastened 5 cm upstream and another 1 cm downstream. A third tile was located 20 cm downstream of the second tile. Three sets of tiles were removed from the stream on each date that fish carcasses were collected. Selected sets of tiles were analyzed for chlorophyll *a* (APHA 1985). In each case, chlorophyll was extracted from the entire tile using 90% acetone.

Results

Gross Appearance

Length of fungal hyphae increased gradually to a maximum of 3–5 mm on approximately day 50 in the spring and day 20 in the summer and then decreased gradually to 0.5 mm by the end of the study. Similarly, percent coverage increased gradually to 100% by day 30 in the spring and day 7 in summer and decreased again as the study progressed. Decomposition seemed to proceed faster in the gut cavity than in the muscle. The skin was intact over the muscle longer than around the abdomen, where rupture and invasion of insects seemed to occur first. The muscle changed in texture from mushy and pastelike to dry and granular, and since the overlying skin remained mostly intact, it is assumed that nutrients leaked out or were absorbed by the fungus on the surface. Skin was first observed to be missing on the head and appeared to have been removed by insects. On the whole it seems that decomposition proceeded from the inside, with organs and muscles gradually collapsing as organic matter decreased. Insects, which were observed on the gills and in the abdominal cavity before being associated with the muscle, may have served as vectors for introduction of microbes.

Periphyton Growth

Apparently, nutrient release from the carcasses was insufficient to stimulate periphyton growth on tiles placed immediately downstream from the decaying fish (Table 1). We presume that nutrient release did occur but that it was at such a low rate that it had no effect on the algae. In streams and lakes

TABLE 1. Mean and standard deviation (in parentheses) of chlorohyll *a* concentration (mg/m^2) on tiles, upstream, "underneath," and downstream of fish carcasses. $N = 3$ except where indicated by an asterisk where $N = 2$.

Date (mo/d/yr)	Upstream	Underneath	Downstream
Spring			
4/9/87	9.31 (2.46)	5.59 (1.86)	8.0 (0.5)
5/1/87	6.98 (2.12)	5.74 (1.22)	8.0 (3.6)
5/9/87	12.42 (1.94)	11.02 (0.71)	11.8 (0.5)
6/6/87	15.52 (3.76)	21.88*	20.8 (7.0)
Summer			
6/24/87	1.55 (0.54)	1.24 (0.54)	1.8 (0.9)
7/1/87	3.88 (0.79)	2.79*	5.2 (0.8)
7/6/87	11.49 (3.17)	13.04 (3.48)	7.7 (1.7)
7/9/87	7.45*	17.23*	9.0 (3.5)
7/13/87	19.09 (4.85)	16.76 (2.66)	11.1 (2.8)
7/16/87	13.27*	13.19 (2.09)	13.6 (2.3)
8/3/87	16.92 (6.66)	11.18 (3.23)	10.5 (3.2)

where large numbers of decaying fish occur, enhanced growth of attached and suspended (phytoplankton) algae has been noted (Juday et al. 1932; Narver 1967; Mathisen 1972; Richey et al. 1975) or direct uptake on nutrients demonstrated (Kline et al. 1990).

Fish Decay Rates

The fish carcasses decayed at reasonably constant rates throughout the two study periods but decomposition was much slower in winter–spring than in summer (Fig. 1). A linear plot gave almost as good a fit as an exponential plot in both winter–spring (correlation coefficient, $r = 0.806$ and 0.866, respectively) and summer ($r = 0.913$ and 0.940, respectively). The linear relationships were chosen over the exponential ones using Occam's rule and because most leaf decay (the only data for freshwater material available for comparison) fits a linear model (Petersen and Cummins 1974). However, the slightly better fit of the exponential line to the data suggests a slowing of the decomposition process in the later stages as a greater percentage of bone and skin constitute the material remaining (winter–spring: $y = 6.839 + 1.261x - (5.352 \times 10^{-3})x^2$; summer: $y = -6.544 - 3.509x - 0.033x^2$). Accumulation of organic matter on the carcasses due to microbial or algal growth was not evident from the data on fish weights after correction for accumulated inorganic sediments (i.e. using derived %OM values). However, such growth may have obscured the actual rate of fish decay, especially midway through each experiment.

By plotting ln (final AFDW/initial AFDW) against time, regression coefficients of -0.0155 and -0.0479 were obtained for winter–spring and summer, respectively. These are the loss rate coefficients, k. The spring value corresponds to a "fast" leaf decomposition coefficient range of 0.010–0.015 (Petersen and Cummins 1974). The summer value was much higher than for most leaf material studied to date (Petersen and Cummins 1974; Oberndorfer et al. 1984; Barnes et al. 1986) but is comparable with summer–fall values for red alder (*Alnus rubra*) in two Alaskan streams (Duncan and Brusven 1985). Given the more suitable nutrient content (e.g. greater content of nitrogen, phosphorus, etc.) of unaltered fish tissue (mainly protein) over that of unaltered leaf or woody material (mainly carbohydrate), the summer decay rates reported here might be expected to approach the upper limit for large particulate organic matter in most temperate mountain streams. Using this same logic, it was surprising that in winter–spring, fish did not decay substantially faster than leaves. Although the rate is much higher than for aspen leaves in East Fork Mink Creek ($k = -0.0026$) (LaPoint 1980), it is comparable with those elsewhere in Idaho (aspen, -0.0114; willow, -0.0125) (Minshall et al. 1982) and Utah (box elder, -0.0269) (Barnes et al. 1986). The mean daily carcass percent loss, calculated from the rate coefficients, using the formula

$$(1 - e^{-k}) \times 100 = \%\ \text{loss/day},$$

gave losses of 1.538 and 4.677%/d for winter–spring and summer, respectively.

Discussion

The values for fish decay reported here exclude the effect of vertebrate scavengers which apparently play a significant role in nature (D. Anderson, Idaho Fish and Game, McCall, ID, and P. Lawson, Idaho State University, pers. comm.; McClelland et al. 1982; Stalmaster and Gessaman 1984; Cederholm et al. 1989). In Mink Creek, tethered (i.e. not protected by screen envelopes) fish disappeared fairly rapidly. In winter–spring, most were present 4 d after placement, but the majority were gone when examined a week later (11 d total). In summer, all tethered fish were gone within 4 d after placement in the stream. It is not possible to say what proportion simply pulled loose from their tethers and washed away compared with those actively removed by scavengers. But indirect evidence, including footprints of medium-sized furbearers and the finding of tags several metres from the stream, indicates that removal by terrestrial vertebrates was at least partially involved. The longest remaining tethered fish (winter–spring: 39 d) was not visible from the surfaceof the stream unlike most of the others, also suggesting that many of the others may have been pulled loose by a visual-type scavenger.

Brusven and Scoggan (1969) have suggested that certain invertebrate (Trichoptera) larvae also may be important in the removal of dead fish from streams, although they apparently only observed the initial stages (first 24 h) of invasion by the caddisflies, by which time only limited penetration of the integument had occurred. Insect scavengers appeared to play an insignificant role in Mink Creek even though they had ready access to and were relatively abundant on the fish carcasses. However, the large, omnivorous Trichoptera observed by Brusven and Scoggan (*Dicosmoecus, Lepidostoma, Psycoglypha*) are rare or absent from Mink Creek (Minshall 1981).

The potential for decomposing fish to collectively influence the dynamics of a riverine ecosystem is quite large. This is particularly evident in the case of anadromous salmonids which

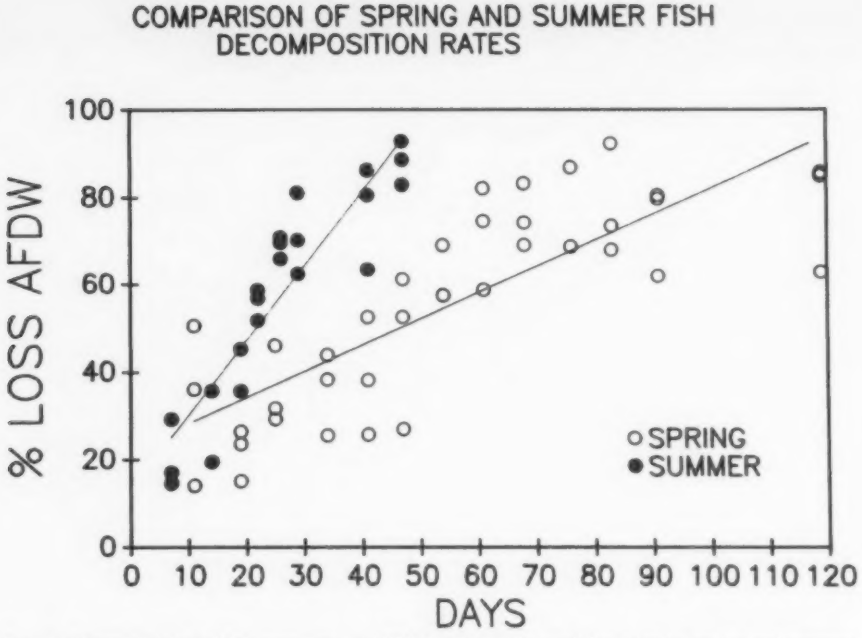


FIG. 1. Decomposition of fish carcasses (%AFDW) enclosed in mesh bags during spring and summer 1987 in West Fork Mink Creek, Bannock County, Idaho, showing a linear fit to the data ($y = 22.344 + 0.597x$, $r = 0.806$; $y = 13.153 + 1.698x$, $r = 0.913$, respectively).

undergo much of their growth and maturation at sea, return to their native stream or lake to spawn, and die en masse after spawning. These fish may be seen as mining or recovering nutrients from areas of accumulation and storage (e.g. the sea), transporting them to nutrient-poor headwater areas, and releasing them to be actively reutilized in a spiraling fashion (Newbold et al. 1981, 1982) down the length of a river ecosystem. Richey et al. (1975) have shown increased phosphate levels in streams containing salmon over those found in salmon-free streams in the same basin. They estimated that a run (14 000 fish) of landlocked sockeye salmon (kokanee, *Oncorhynchus nerka*) was capable of raising the phosphate content of a small tributary stream 4–6 μg/L and contributing nearly 44.6 kg of phosphorus to the stream. On a larger scale, Juday et al. (1932) estimated that sockeye salmon add in excess of 2×10^6 kg of decomposable organic matter and 5000 kg of phosphorus to the Karluk River system in Alaska in an average year. Among other things, such refertilization has the potential for stimulating or maintaining fish production in the affected lakes and streams (Donaldson 1967; Mathisen 1972).

In the case of streams and lakes receiving large inputs of nutrients from fish carcasses, algae apparently serve as important mediators in the downstream transport of nutrients (Mathisen 1972; Richey et al. 1975; Kline et al. 1990). The periphyton thus act as a reservoir, holding the nutrients in the spawning stream to be released after the final decay of the fish. However, in nutrient-poor systems or cases of isolated carcasses of small fish or other animals, as in Mink Creek, nutrient uptake appears to be restricted to the microheterotrophs directly associated with the carcass or direct ingestion of decaying tissue by macroinvertebrate consumers. In such cases the enhancement of algal production or downstream transport of detectable amounts of nutrient appears absent or minor, suggesting extremely tight nutrient spirals and high nutrient retention (Minshall et al. 1985).

Acknowledgments

This research was supported by grant No. 599 from the Faculty Research Committee, Idaho State University. C. Nelson and C. T. Robinson made major contributions to several aspects of the study. R. Arbon, S. Danamraj, M. Haslett, B. Jamison, P. Koetsier, and L. Van Every helped with the initial placement of fish in Mink Creek and with laboratory processing of collected materials. D. E. Lawrence determined the temperature means, ranges, and degree-days from strip chart tracings. V. Moore, Idaho Fish and Game Department, was instrumental in obtaining the trout used in the study.

References

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 1985. Standard methods for the examination of water and wastewater. APHA, Washington, DC.

BARNES, J. R., J. V. MCARTHUR, AND C. E. CUSHING. 1986. Effect of excluding shredders on leaf litter decomposition in two streams. Great Basin Nat. 46: 204–207.

BRUSVEN, M. A., AND A. C. SCOGGAN. 1969. Sarcophagous habits of Trichoptera larvae on dead fish. Entomol. News 80: 103–105.

CEDERHOLM, C. J., D. B. HOUSTON, D. L. COLE, AND W. J. SCARLETT. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Can. J. Fish. Aquat. Sci. 46: 1347–1355.

DONALDSON, J. R. 1967. The phosphorus budget of Iliamna Lake, Alaska, as related to the cyclic abundance of sockeye salmon. Ph.D. Thesis, University of Washington, Seattle, WA. 141 p.

DUNCAN, W. F. A., AND M. A. BRUSVEN. 1985. Energy dynamics of three low-order southeast Alaska streams: allochthonous processes. J. Freshwater Ecol. 3: 233–248.

HALL, C. A. S. 1972. Migration and metabolism in a temperate stream ecosystem. Ecology 53: 585–604.

JUDAY, C., W. H. RICH, G. I. KEMMERER, AND A. MANN. 1932. Limnological studies of Karluk Lake, Alaska 1926–1930. Bull. U.S. Bur. Fish. 47: 407–436.

KLINE, T. C. JR., J. J. GOERING, O. A. MATHISEN, P. H. POE, AND P. L. PARKER. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Can. J. Fish. Aquat. Sci. 47: 136–144..

LAPOINT, T. W. 1980. The role of ciliated protozoa and bacteria in stream benthic organic matter decomposition. Ph.D. dissertation, Idaho State University, Pocatello. ID.

MATHISEN, O. A. 1972. Biogenic enrichment of sockeye salmon lakes and stock productivity. Verh. Int. Ver. Limnol. 18: 1089–1095.

MCCLELLAND, B. R., L. S. YOUNG, D. S. SHEA, P. T. MCCLELLAND, H. L. ALLEN, AND E. B. SPETTIGNA. 1982. The bald eagle concentrations in Glacier National Park, Montana: origin, growth, and variation in numbers. Living Bird 19: 133–155.

MINSHALL, G. W. 1981. Structure and temporal variations of the benthic macroinvertebrate community inhabiting Mink Creek, Idaho, U.S.A., a 3rd order Rocky Mountain stream. J. Freshwater Ecol. 1: 13–26.

MINSHALL, G. W., J. T. BROCK, AND T. W. LAPOINT. 1982. Characterization and dynamics of benthic organic matter and invertebrate functional feeding group relationships in the Upper Salmon River, Idaho (USA). Int. Rev. Gesamten Hydrobiol. 67: 793–820.

MINSHALL, G. W., K. W. CUMMINS, R. C. PETERSEN, C. E. CUSHING, D. A. BRUNS, J. R. SEDELL, AND R. L. VANNOTE. 1985. Developments in stream ecosystem theory. Can. J. Fish. Aquat. Sci. 42: 1045–1055.

NARVER, D. W. 1967. Primary productivity in the Babine Lake system, British Columbia. J. Fish. Res. Board Can. 24: 2045–2052.

NEWBOLD, J. D., J. W. ELWOOD, R. V. O'NEILL, AND W. VAN WINKLE. 1981. Measuring nutrient spiralling in streams. Can. J. Fish. Aquat. Sci. 38: 860–863.

NEWBOLD, J. D., R. V. O'NEILL, J. W. ELWOOD, AND W. VAN WINKLE. 1982. Nutrient spiralling in streams: implications for nutrient limitation and invertebrate activity. Am. Nat. 120: 628–652.

OBERNDORFER, R. Y., J. V. MCARTHUR, J. R. BARNES, AND J. DIXON. 1984. The effect of invertebrate predators on leaf litter decomposition in streams. Ecology 65: 1325–1331.

PETERSEN, R. C., AND K. W. CUMMINS. 1974. Leaf processing in a woodland stream. Freshwater Biol. 4: 343–368.

RICHEY, J. E., M. A. PERKINS, AND C. R. GOLDMAN. 1975. Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream. J. Fish. Res. Board Can. 32: 817–820.

STALMASTER, M. V., AND J. A. GESSAMAN. 1984. Ecological energetics and foraging behavior of overwintering bald eagles. Ecol. Monogr. 54: 407–428.

Stream Ecosystem Recovery Following a Catastrophic Debris Flow[1]

Gary A. Lamberti,[2] Stanley V. Gregory, Linda R. Ashkenas, Randall C. Wildman, and Kelly M. S. Moore

Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA

Lamberti, G. A., S. V. Gregory, L. R. Ashkenas, R. C. Wildman, and K. M. S. Moore. 1991. Stream ecosystem recovery following a catastrophic debris flow. Can. J. Fish. Aquat. Sci. 48: 196–208.

We studied recovery processes for 3 yr in Quartz Creek (Cascade Mountains, Oregon), a third-order stream catastrophically impacted by a February 1986 debris flow for which both predisturbance data and an upstream control reach were available. The debris flow altered channel geomorphology and destroyed riparian vegetation for 500 m, resulting in a reach with short, disordered channel units, low hydraulic retention, and an open canopy. High irradiance levels and reduced grazing by macroinvertebrates contributed to rapid accrual of benthic algae in the disturbed reach, which formed the bioenergetic basis for ecosystem recovery. Macroinvertebrates (mostly herbivores) recovered to upstream densities and taxonomic richness within 1 yr, although effects on community structure persisted into the second year. Cutthroat trout (*Oncorhynchus clarki*) populations were locally decimated by the disturbance, but by the following year, recruitment of young-of-the-year trout into the reach exceeded that of the upstream reach and populations had recovered to predisturbance densities. Despite the general rapid recovery of the biota within the disturbed reaches, most populations showed broad temporal fluctuations in abundance, suggesting that ecosystem stability was diminished by the debris flow. Long-term monitoring of Quartz Creek may yield additional insight into the role of episodic disturbance in stream ecosystems.

Nous avons étudié durant 3 ans les processus de rétablissement du ruisseau Quartz, situé dans les montagnes Cascade en Oregon, un cours d'eau de troisième ordre durement touché par une coulée de débris en février 1986. Des données sur ce cours d'eau prises avant cet événement de même que l'existence d'un tronçon non touché situé en amont pouvant servir de témoin ont facilité cette étude. La coulée de débris a transformé la géomorphologie du lit et a détruit la végétation riparienne sur 500 m; dans une partie du cours d'eau, elle a ainsi laissé une série de courts canaux désorganisés, réduit passablement la rétention hydraulique et dévasté le couvert végétal. Les taux élevés de rayonnement solaire et la diminution du broutage par les macroinvertébrés ont contribué à l'accroissement rapide de la quantité d'algues benthiques dans le tronçon perturbé; ces algues ont joué un rôle bioénergétique clé dans le rétablissement de l'écosystème. Les macroinvertébrés (surtout herbivores) ont retrouvé en une année les mêmes densités et la même diversité taxonomique que dans le tronçon témoin situé en amont, mais la structure des communautés n'était pas encore rétablie après la première année. En certains endroits, les populations de truite fardée (*Oncorhynchus clarki*) ont été décimées par cette perturbation, mais l'année suivante, le recrutement de jeunes de l'année y était supérieur à celui du tronçon témoin, produisant une densité de population équivalente à celle d'avant la coulée de débris. Bien que de façon générale le biote des secteurs touchés s'est rétabli rapidement, les abondances de la plupart des populations ont montré des fluctuations temporelles importantes, ce qui laisse entendre que la stabilité de l'écosystème a été réduite. La surveillance à long terme du ruisseau Quartz pourrait nous fournir d'autres renseignements sur les effets des perturbations occasionnelles sur l'écosystème des cours d'eau.

Received November 22, 1989
Accepted August 10, 1990
(JA369)

Reçu le 22 novembre 1989
Accepté le 10 août 1990

Disturbance is a major factor determining the physical and biological organization of ecosystems (Connell 1978; Pickett and White 1985). A disturbance generally is defined as a discrete event that disrupts population, community, or ecosystem structure, often by changing resource abundance or the physical environment (Sousa 1984; Resh et al. 1988). In stream ecosystems, natural disturbance often is generated by floods of sufficient magnitude to scour attached microbiota from sediments (Fisher et al. 1982), remove or kill benthic macroinvertebrates (Siegfried and Knight 1977), and displace fish and other vertebrates (Meffe 1984). However, because annual flooding is a normal event in most streams, many lotic organisms are adapted to this regular disturbance through behavioral features, life-history adjustments, or reproductive traits (Resh et al. 1988).

Catastrophic disturbance, such as severe hurricanes or intense wildfires, may overwhelm biotic resistance and elicit profound changes in ecosystem structure and function (Connell 1978). In streams, catastrophic disturbance is exemplified by debris flows (sometimes called debris torrents), which are mass movements of sediment and debris down stream channels that greatly alter both the streambed and adjacent riparian zone (Swanson et al. 1987). Unlike floods, which occur with some regularity in most streams (e.g. McElravy et al. 1989), debris flows are rare, unpredictable events (>50 yr recurrence interval; Swanson et al. 1987) that can severely affect aquatic biota.

[1]Technical paper No. 9331 of the Oregon Agricultural Experiment Station.

[2]Present address: Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA.

In montane regions of the Pacific Northwest, major rainstorms can trigger landslides that deliver large pulses of sediment and debris to stream channels (Swanston and Swanson 1976; Swanson and Lienkaemper 1978). Landslides that enter small, steep tributaries often create a slurry of soil, water, boulders, and woody debris that travels down the channel as a debris flow. Debris flows may then enter mainstem reaches, often increasing in size as they mobilize materials already present in the channel. These flows can exceed 10 000 m^3 in volume and travel distances ranging from metres to kilometres, depending on local conditions, at speeds greater than 10 m/s (Swanson et al. 1987). Debris flow usually stop where the channel widens, the gradient declines, or constrictions impede their movement, often terminating in a large accumulation of debris.

Although landslides typically affect <1% of a watershed in a particular episode, debris flows may influence >10% of the channel network because of their ability to move down stream courses (Swanson et al. 1987). Debris flows may scour channels down to bedrock, rearrange the existing streambed, or deposit new material on top of older sediment. Consequently, debris flows are major disturbances worldwide for streams in steep terrain or unstable geology (Swanson et al. 1987). In the forested landscapes of the Pacific Northwest, debris flow frequencies have increased due to logging, road and landing construction, inadequate road drainage, and other land-use practices that reduce hillslope stability and accelerate mass wasting (Swanson et al. 1987). However, the direct effects of debris flows on aquatic and riparian resources rarely have been evaluated.

In this paper, we report the effects of a debris flow on important physical, chemical, and biological attributes of Quartz Creek, a third-order stream in the Cascade Mountains of Oregon, USA. This stream had been studied intensively several years prior to the event and thus predisturbance information existed to help assess disturbance effects and rates of ecological recovery. Our objectives were to (1) document ecosystem recovery for 3 yr following the debris flow, (2) compare pre- and postdisturbance conditions in Quartz Creek, (3) relate patterns of biotic recovery to physical features of the environment, and (4) evaluate factors controlling rates of ecosystem recovery.

Study Area

Quartz Creek is a third-order stream located in the McKenzie River drainage on the west slope of the Cascade Mountains in Oregon and adjacent to the H. J. Andrews Experimental Forest (Fig. 1). The stream drains a 12-km^2 basin of mixed second-growth forest of Douglas-fir (*Pseudotsuga menziesii*) and red alder (*Alnus rubra*). Most of the lower basin was salvage logged in the 1940's following wildfire, although some recent harvest has occurred in upper portions of the basin. Quartz Creek flows approximately 6 km with an average gradient of 5% before emptying into Blue River Reservoir. Baseflow discharge ranges from about 0.05 m^3/s in summer to about 1.0 m^3/s in winter. Elevation varies from 370 to 1070 m within the basin and from 500 to 550 m at the study sites.

From 20 to 23 February 1986, the Quartz Creek basin received about 25 cm of rain in a 96-h period. The freezing level rose simultaneously and the intense rains melted much of the 25- to 50-cm snowpack in the area. In the early morning of 23 February 1986, the combined rain and snowmelt triggered a landslide in a clearcut near a first-order tributary to Quartz Creek (Fig. 1). The slide spilled into the tributary, traveled about 500 m down the tributary, and swept into Quartz Creek, entraining additional woody debris, sediment, and water from the channel as it moved. Total mass of the debris flow was estimated at 5000 m^3, which traveled at a velocity of between 5 and 10 m/s. The flow traveled 330 m in Quartz Creek before lodging as an accumulation of wood and sediment that spanned the stream channel. This debris dam measured about 40 m in length, 25 m in width, and 5 m in height and contained about 700 pieces of wood. A reach of about 200 m downstream of the debris dam was affected by the leading edge of the debris flow before the accumulation formed.

Upstream portions of Quartz Creek that were not affected by the debris flow experienced a major flood on 22–23 February 1986. Although Quartz Creek is not gauged for discharge, nearby stations provided some indication of flood magnitude. Two adjacent gauged streams, Lookout Creek and Blue River, had peak discharges of an estimated 8- to 10-yr average recurrence interval, based on 25 yr of hydrologic record (Friday and Miller 1984). The average return interval for the debris flow was much longer, probably in excess of 50 yr (Swanson et al. 1987).

We designated three reaches in Quartz Creek for intensive study (Fig. 1): (1) a reach *upstream* of both the tributary described above and a second tributary that had a smaller debris flow, which experienced only flooding on 22–23 February 1986, (2) the *debris flow* reach that was severely affected by both the debris flow and the flood, and (3) a reach *downstream* of the debris accumulation that was flooded and received moderate disturbance from sediment and debris that swept through before the accumulation stabilized. Reach lengths were 184 m (upstream), 276 m (debris flow), and 176 m (downstream). By chance, the debris flow occurred in a portion of Quartz Creek that had been studied during 1982–83 (Speaker 1985; Moore 1987). These previous studies provided physical and biological data on conditions prior to the debris flow.

Materials and Methods

Sampling Schedule

Water chemistry, algae, detritus, invertebrates, and fish were sampled in the three study reaches at 3- to 5-mo intervals between March 1986 and November 1988. We continue to take semiannual samples from Quartz Creek as part of the H. J. Andrews Long-Term Ecological Research Program. The sampling schedule was designed to represent seasonal conditions important to stream biota: late winter following high discharge, summer low flow period, and autumn detrital loading. Sampling was more frequent in the first year after the event to document the rapid physical and biological changes that often occur shortly after disturbance. On the first sampling date (26 March 1986), we did not sample the downstream reach; this site was added on the second sampling date (24 April 1986). Additional physical and biological parameters, including channel morphology, irradiance, hydraulics, and leaf abundance, were measured less frequently.

Physical Parameters

Geomorphology

Stream *reaches* in Quartz Creek were distinguished based on the degree of large-scale geomorphic disturbance described above. The stream channel and adjacent riparian surfaces were mapped for each reach during summer 1986. A 108-m segment

FIG. 1. Location of study sites in Quartz Creek, Oregon, USA. Dotted areas along Quartz Creek and its tributaries indicate zones of 1986 debris flow influence. DF = debris flow; DD = debris dam.

of the debris flow reach was mapped in 1983. Within the wetted channel of each reach, we determined the sequence and surface dimensions of its *channel units*. Channel units are distinct hydraulic and geomorphic features, formed during high flow, that span the channel and typically have lengths equal to several channel widths. Channel units given in increasing gradient are pools, riffles, rapids, cascades, bedrock falls, and boulder steps (see Grant 1986 and Gregory et al. 1991 for further description). For each channel unit, we then visually estimated the percent surface area of *subunits*. Subunits are discrete patches comprising each unit that are controlled by local structural and hydraulic features; these include stream margins, backwaters, secondary channels, and boulder eddies (grouped into "edge" habitats), pools ("slow-water" habitats), and riffles, chutes, and hydraulic jumps (grouped into "fast-water" habitats).

Irradiance

Light energy in the three study reaches was measured periodically from 1986 to 1988 with a Li-Cor quantum radiometer held at the water surface. Three stations within each reach were measured repeatedly.

Water chemistry

Water samples were collected on 13 occasions during 1986–88 from the three reaches and from the tributary where the debris flow started. Samples were filtered in the field, placed on ice, and analyzed within 48 h of collection. Chemical analyses focused on inorganic and organic forms of nitrogen and phosphorus. Ammonium nitrogen (NH_4-N) was analyzed by the hypochlorite oxidation method, and nitrate plus nitrite nitrogen (NO_3-N + NO_2-N) was measured by the cadmium reduction technique (APHA et al. 1975). Reduced nitrogen was analyzed by Kjeldahl digestion and subsequent analysis of ammonium. Total dissolved nitrogen was calculated as Kjeldahl N + NO_3-N + NO_2-N. Orthophosphate (PO_4-P) was determined colorimetrically by the ammonium molybdate reaction, and total phosphorus was analyzed by digestion and analysis as orthophosphate (APHA et al. 1975).

Hydraulics

We measured hydraulic features of Quartz Creek with fluorescent dye tracers. We released a known amount of fluorescein dye (generally 5 g/L, w/v) into a turbulent area of the channel. Water samples were collected 50 or 100 m downstream at 15- to 60-s intervals, depending on discharge. Dye concentration in each sample was measured with a fluorometer. Discharge was calculated based on dye dilution (Replogle et al. 1976). Hydraulic residence time (HRT) was calculated as the time required for 95% of the dye to move through the reach.

On 18 March 1987, we released dye into the upstream reach, the debris flow reach, and through the debris dam. Dye releases were conducted sequentially upstream to prevent contamination of downstream study reaches. An additional five dye releases were conducted in different sections of the debris flow reach during both summer and winter baseflow, 1986–88. Predisturbance data were provided by nine dye releases conducted in the debris flow reach during 1983 (Speaker 1985).

Biological Parameters

Organic matter

Benthic detritus was sampled using an enclosed McNeil sampler (123 cm^2) driven 10 cm into the streambed. Contents of the sampler were evacuated and all detritus larger than 0.7 μm was retained using a combination of sieves and filters (Whatman GF/F). To determine ash-free dry mass (AFDM) of detritus, each sample was dried for 24 h at 55°C, weighed, combusted for 12 h at 500°C, and reweighed. On each sampling date within each reach, we collected five samples from each of riffle (= fast-water), pool (= slow-water), and backwater (= edge) habitats in a stratified random sampling design (N = 15 for each reach). Care was taken to distribute the samples over the entire length of each reach. Using the habitat proportions generated

TABLE 1. Amounts and proportions of stream subunits for the three study reaches of Quartz Creek in July 1986. Subunit areas were determined for reaches of 184 m (upstream), 276 m (debris flow), and 176 m (downstream). These percentages were used to calculate weighted means and variances for benthic detritus and macroinvertebrates.

Subunit	Upstream	Debris flow	Downstream
Fast-water habitat			
Total area (m^2)	411.2	548.1	407.0
Area/100 m	233.5	198.6	220.8
Percent area	53.4	50.4	53.9
Slow-water habitat			
Total area (m^2)	235.7	342.3	204.5
Area/100 m	133.8	124.0	111.0
Percent area	30.6	31.5	27.1
Edge habitat			
Total area (m^2)	123.2	197.1	143.5
Area/100 m	70.0	71.4	77.9
Percent area	16.0	18.1	19.0

from subunit estimations (Table 1), we calculated a weighted average detritus load ($\bar{x}_w$) and variance of the mean (s_w^2) for each reach (simplified from Krebs 1989) as

$$\bar{x}_w = \sum_{i=1}^{3} p_i \bar{x}_i$$

and

$$s_w^2 = \sum_{i=1}^{3} p_i^2 s_i^2/n_i$$

where p_i is the proportion of the total reach represented by habitat i, $\bar{x}_i$ and s_i^2 are the observed mean and variance for habitat i, and n_i is the sample size for habitat i. The standard error of the weighted mean is calculated as s_w (Krebs 1989).

On 18 November 1987, we measured the densities of fallen leaves within the active stream channel. Five 1-m-wide transects were established perpendicular to the channel at random locations within each reach. Leaves were counted in every 1-m^2 quadrat along each transect while noting if the quadrat sampled wetted channel or nonwetted active channel (=active channel shelf).

Benthic algae

Standing crop of benthic algae was measured by extraction of the pigment chlorophyll *a*, an indicator of algal abundance. Five sets of three rocks (10–20 cm in diameter) were randomly collected from riffles of each reach on each sampling date. Rock sets were bagged, placed on ice in the field, and frozen within 8 h. Within 4 wk of collection, rocks were soaked in buffered 90% acetone for 24 h. Extracted chlorophyll *a* was measured with a spectrophotometer using the trichromatic method (Strickland and Parsons 1968). Rock surface area was determined by wrapping each rock in aluminum foil, weighing the foil, and estimating area from known areal weight of foil. This estimate was halved to adjust for surface area exposed to solar radiation (normally about 50% of the rock).

Macroinvertebrates

Benthic macroinvertebrates were sampled using a modified Hess sampler (510 cm^2) with 250-μm-mesh screen. On each sampling date, 15 Hess samples were collected from each reach using the same stratified random sampling approach described for detritus. Samples were preserved immediately in 95% ethanol and later sorted in the laboratory. Samples visually estimated to exceed 2000 animals were subsampled (1/4 or 1/8) using a sample splitter. All invertebrates were enumerated and identified to the lowest taxonomic level possible (usually genus), except for Chironomidae and Oligochaeta, which were not identified to lower levels. Weighted average density and variance of macroinvertebrates were calculated for each reach as for detritus.

Fish populations

Only one species of fish, cutthroat trout (*Oncorhynchus clarki*) (formerly *Salmo clarki*), was found in Quartz Creek. Stream lengths of 150 m (upstream and downstream reaches) and 250 m (debris flow reach) were repeatedly sampled for trout. To determine trout population density, trout were counted by direct observation while snorkeling in March and June 1986 only whereas double-pass electroshocking was used for all subsequent estimates. During snorkeling, a diver swam upstream from the bottom to the top of the reach recording the number of trout. Fish were assigned to fry (0+ yr or young-of-the-year) or adult (1+ yr and older) age classes based on size. A second diver repeated this procedure about 1 h later. The mean of the two observations was used in population estimates; no verification with electrofishing was conducted on these two dates. During electroshocking on all later dates, sequential 50-m lengths were blocked with nets at both ends and fish were sampled using the double-pass method (Armour et al. 1983). All fish were anesthetized, measured for total length (±1 mm), weighed for wet mass (±0.05 g), revived, and returned to the reach. In June 1987 and June 1988, newly emerged fry were not electroshocked because their small size made them highly sensitive to shocking. However, because young fry are found almost exclusively along shallow stream margins (Moore and Gregory 1988a), we made visual fry counts within each reach. One observer walked upstream along each streambank recording the number of fry; these are probably underestimates of true fry numbers.

Statistical Analyses

Several statistical methods were used to analyze physical and biological data. For nonrepeated measures, we used analysis of variance (ANOVA) and the Student–Newmann–Keuls (SNK) multiple range procedure on log-transformed data to test for differences in means among the three reaches. For repeated measures and inventories, graphical methods were used to reveal temporal trends in abundance. Size–frequency plots of trout populations were used to follow cohorts over time. Regression techniques were used to analyze some parameters such as hydraulics. For all tests, differences were considered significant at the $p<0.05$ level. It should be noted that because reaches were not replicated (i.e. one debris flow was studied), these tests only establish statistical differences among reaches and we cannot attribute differences solely to the disturbance.

Results

Initial Observations

The upstream reach of Quartz Creek experienced a flood on 22–23 February 1986, but physical alteration of the site was minimal in comparison with downstream reaches subjected to the debris flow. Dense riparian vegetation, large boulders, and wood debris contributed to a heterogeneous stream channel

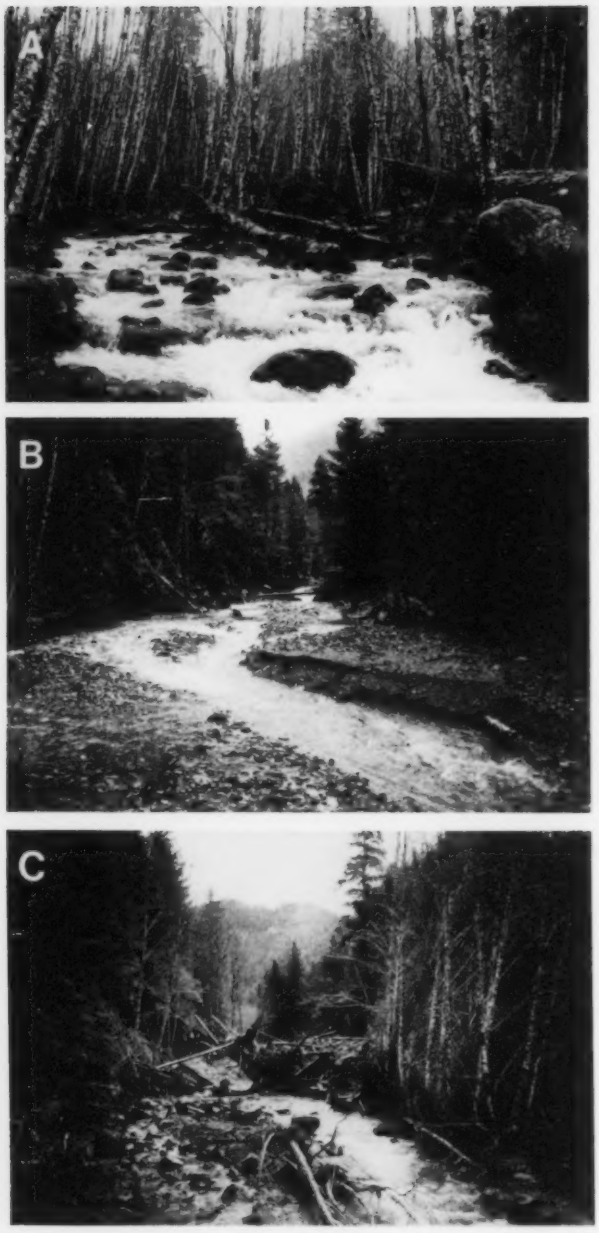


FIG. 2. Photographs of three study reaches in Quartz Creek taken approximately 1 mo after the debris flow. (A) Upstream reach, showing alder canopy and high channel roughness; (B) debris flow reach, showing open canopy and low channel complexity; (C) downstream reach, showing debris dam and open canopy.

in the upstream reach (Fig. 2A). Many aquatic organisms probably survived the flood alone.

In the debris flow reach, our qualitative observations 48 h after the flood and debris flow indicated severe physical changes including canopy removal, loss of wood debris, and reorganization of channel sediments (Fig. 2B). The aquatic fauna and flora probably were completely lost from the reach. Even 2 d after the event, landslide sediment continued to enter Quartz Creek from the tributary, which scoured the streambed or buried substrates. At that time, we found no macroinvertebrates and no macroscopic algal growth on the surfaces of stones in the debris flow reach. We assume that all fish in the debris flow reach were killed or displaced downstream; dead trout were found high on the streambanks downstream.

Riparian vegetation of the downstream reach was removed or battered by the leading edge of the debris flow, but some channel complexity was retained by existing and delivered wood and boulders (Fig. 2C). A portion of the stream biota probably survived in this reach but ecological recovery of the disturbed reaches began with a greatly diminished aquatic biota.

Response in Physical Features

Channel geomorphology

The channel unit sequence in the upstream reach was characterized by alternating cascades (25–40 m long) and pools (5–10 m long) arranged in a stairstep fashion, with few riffles or rapids (Fig. 3). This pattern is typical of many high-gradient streams in the Cascade Mountains (Sullivan et al. 1987).

The debris flow substantially affected channel morphology of Quartz Creek. Prior to the disturbance, the upper 108 m of the debris flow reach was composed predominantly of short pools (38% of reach length) and cascades (37%), with a shorter distance of riffles and rapids (23%) (Fig. 3). After the debris flow, the reach had a disordered sequence of channel units. Pools in the upper 108 m were reduced to 14% of reach length and riffle/rapid distance was increased to 43%. In the lower part of the debris flow, channel units were longer and included several large pools formed in deep sediments trapped behind the debris accumulation. The downstream reach, which was partially buffered by the debris dam, was composed mostly of long rapids and cascades, with only two pools.

Stream gradient in the debris flow reach before the disturbance was 5.3% but declined to 3.4% after the debris flow; this is due mainly to deposition of 2–6 m of sediment and wood in and behind the debris dam, which reduced overall reach gradient. Gradient in the upstream reach was 6.3%, which was similar to that in the debris flow reach before the event. The downstream reach had a gradient of 3.2% after the disturbance, possibly also associated with sediment deposition.

Despite disturbance-induced changes in the channel unit sequence and in gradient, all three reaches contained similar proportions of subunit habitats (Table 1). In all reaches, fast-water habitat comprised 50–54% of total reach area, pool habitat formed 27–32% of the area, and edge habitat was 16–19%. Thus, subunit quantities indicated a high degree of reach similarity in aquatic habitat, even soon after the debris flow. However, there were qualitative differences in habitat. For example, edge habitat in the disturbed reaches was mostly associated with deep eddies created around large particles in midchannel areas, such as boulders delivered by the debris flow. In the upstream reach, much of the edge habitat occurred along stream margins, such as in shallow backwaters, within side channels, and around wood debris.

Riparian characteristics

Prior to the debris flow, all three reaches had closed riparian canopies dominated by 40- to 50-yr-old alder trees and diverse understory vegetation that heavily shaded the stream. The debris flow removed a 5- to 15-m swath of trees and understory plants on each side of the stream along the entire length of both the

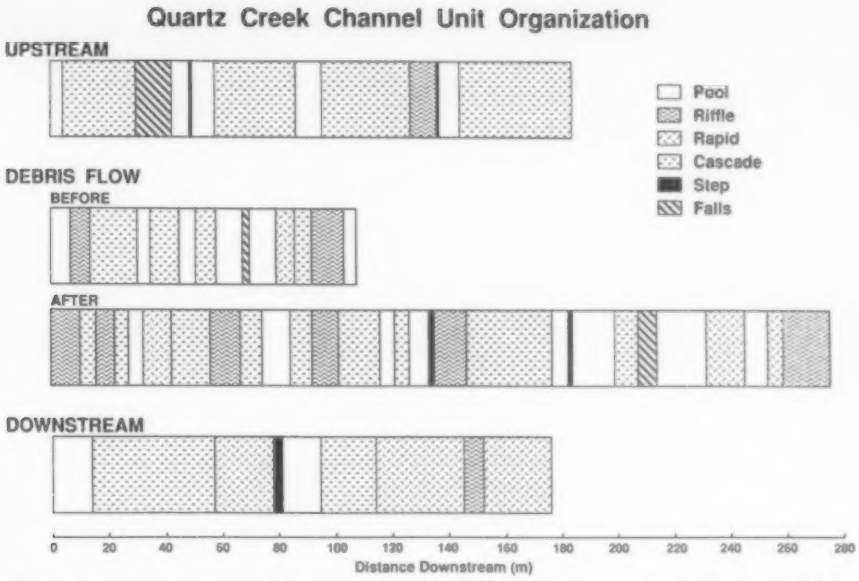


FIG. 3. Sequence of channel units in the three study reaches of Quartz Creek following the flood and debris flow of 1986. The before-disturbance (1983) sequence for the upper 108 m of the debris flow reach is presented for comparative purposes. Lateral dimensions of the channel units are not drawn to scale.

TABLE 2. Concentrations of selected nutrients in the three study reaches and tributary of Quartz Creek during 1986–88. Mean ± SE and range (in parentheses) are expressed in μg/L; $N = 11–13$.

Nutrient	Upstream	Debris flow	Downstream	Tributary
Total P	20 ± 1 (15–27)	20 ± 2 (13–33)	19 ± 1 (12–30)	30 ± 3 (18–49)
PO_4-P	10 ± 1 (8–13)	11 ± 1 (8–14)	10 ± 1 (7–13)	16 ± 1 (7–20)
Total N	24 ± 4 (5–65)	28 ± 3 (7–50)	26 ± 7 (7–46)	43 ± 8 (18–114)
Organic N	17 ± 5 (0–63)	18 ± 3 (2–34)	15 ± 2 (1–29)	23 ± 4 (4–60)
NH_4-N	3 ± 1 (1–5)	3 ± 1 (2–8)	3 ± 1 (0–6)	4 ± 1 (1–7)
NO_3-N + NO_2-N	4 ± 1 (1–11)	6 ± 1 (2–17)	7 ± 2 (1–18)	16 ± 5 (1–49)

debris flow and downstream reaches. This resulted in a 15- to 40-m-wide canopy opening (including the stream channel) in the two disturbed reaches, which greatly increased solar radiation reaching the stream but potentially reduced detritus loading.

Irradiance at the stream surface was substantially higher in the disturbed reaches that in the upstream reach. For example, photon flux density was generally less than 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the upstream reach, compared with 500–2500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the debris flow and downstream reaches at the same time. These large differences in irradiance were significant (Wilcoxon $p < 0.05$; $N = 12$) and persisted throughout the 3-yr study of Quartz Creek. By 1988, however, alder seedlings were growing on most riparian surfaces within the disturbed reaches and beginning to shade stream margins.

Water chemistry

In general, nutrient concentrations were consistently low in all three study reaches of Quartz Creek (Table 2). Orthophosphate (PO_4-P) concentration averaged about 10 μg/L in all three reaches and total dissolved phosphorus was about 20 μg/L. Concentrations of inorganic nitrogen forms also were low in Quartz Creek, which is typical of streams in the Cascade Mountains. Ammonium (NH_4-N) generally was less than 5 μg/L in all reaches. Nitrate plus nitrite (NO_3-N + NO_2-N) averaged 4 μg/L in the upstream reach, but was slightly higher in the debris flow and downstream reaches at 6–7 μg/L. Levels of total nitrogen and total organic nitrogen were less than 30 μg/L in all three reaches of Quartz Creek.

Nutrient concentrations were generally 25–50% higher in the tributary where the debris flow began than in Quartz Creek (Table 2), perhaps due to release of nutrients from the clearcut and landslide area coupled with diminished vegetative uptake of those nutrients. Higher nitrate concentrations in the tributary may also reflect increased nitrification associated with greater supply of dissolved organic nitrogen; however, nutrient contribution by the tributary to Quartz Creek was probably minor because tributary discharge (<0.01 m^3/s) was low relative to Quartz Creek (>0.05 m^3/s).

Hydraulic retention

Dispersion and movement rates of fluorescein dye in Quartz Creek provided information on reach hydraulics. Hydraulic residence time (HRT), a relative index of reach heterogeneity, was calculated as the time required for 95% of the dye to move through a designated reach. Over 50-m reaches, HRT was 3.1 min in the debris flow reach compared with 4.8 min in the upstream reach (Fig. 4A), indicating lower hydraulic retention in the disturbed reach. Dye released through the complex debris dam indicated long hydraulic retention, with a 95% passage time of 23.4 min.

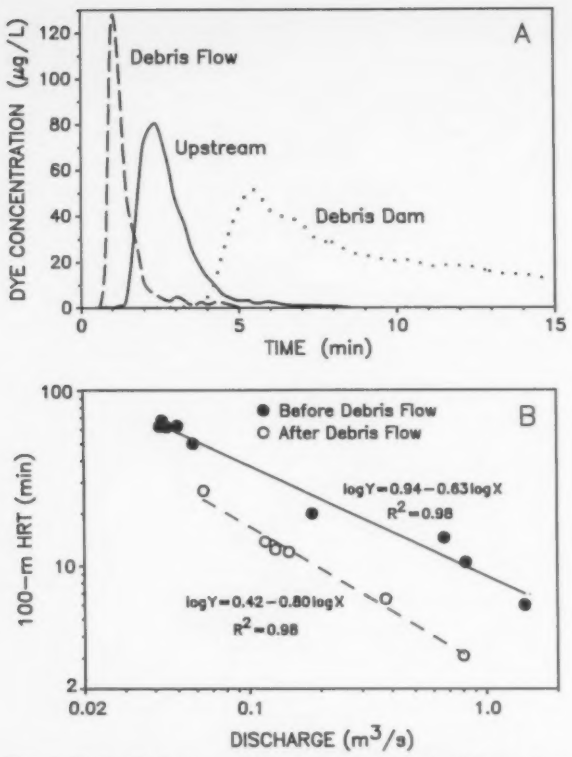


FIG. 4. Hydraulic features of Quartz Creek, determined from releases of fluorescein dye into the channel. (A) Dye concentration over time at 50 m downstream of the release point for the debris flow and upstream reaches, and within the debris accumulation; (B) 95% hydraulic residence time (HRT) for 100-m reaches in the debris flow reach, before and after the disturbance, at different discharges.

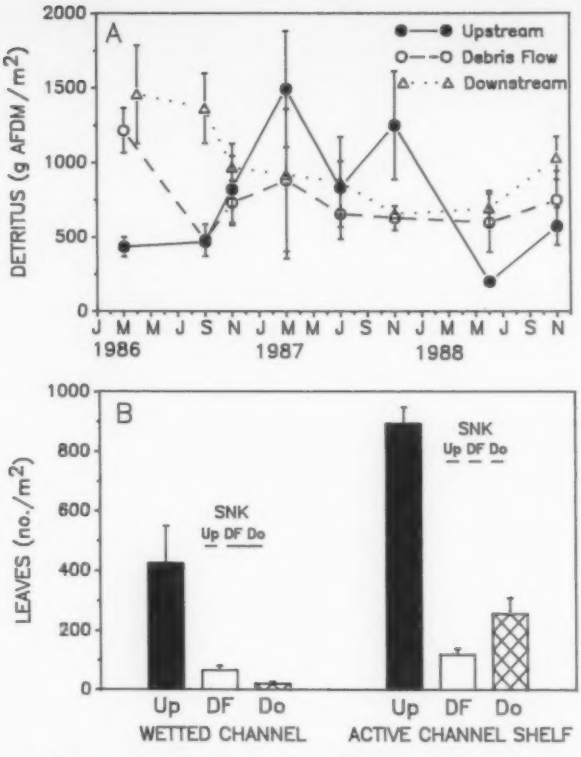


FIG. 5. Mean abundance (± SE) of detritus in the three study reaches of Quartz Creek. (A) Standing crop of benthic detritus from 1986 to 1988; (B) density of fallen leaves in the wetted channel and on the active channel shelf in November 1987. SNK = results of a Student–Newmann–Keuls test where different underscores indicate significantly different mean densities ($p<0.05$).

Using data from a series of 100-m dye releases conducted in 1983 and 1986–88, HRT was compared with discharge in the debris flow reach before and after the disturbance (Fig. 4B). The debris flow reduced HRT to about one third of the predisturbance residence times over a range of discharges. Logarithmic regression analysis indicated that although the slopes were similar, the HRT intercept value before the event (a = 0.94) declined substantially after the debris flow (a = 0.42). Thus, the debris flow substantially reduced HRT at all discharges, apparently by simplifying channel structure and eliminating large pools.

Response in Biological Features

Benthic detritus

The mean abundance of benthic detritus generally varied from 0.5 to 1.5 kg/m^2 in all three study reaches of Quartz Creek (Fig. 5A). Detritus was about 0.5 kg/m^2 in the upstream reach for most of 1986, probably because the severe flood of February 1986 removed stored detritus. From autumn 1986 to autumn 1988, however, the upstream reach showed marked seasonal fluctuations of up to 1 kg/m^2 in mean detrital standing crop, probably reflecting seasonal loading and removal of detritus. Detritus appeared to increase in the autumn with input of deciduous leaves and decline from winter through summer as organic matter was removed by high flow, decomposition, or consumption.

In contrast, the debris flow and downstream reaches showed little seasonality in detrital standing crops (Fig. 5A). Detritus was abundant at >1 kg/m^2 in the disturbed reaches soon after the disturbance, probably the result of delivery of organic matter by the debris flow. After autumn 1986, detritus declined to relatively constant levels near 0.6 kg/m^2, probably because streamside sources of deciduous vegetation were removed by the debris flow while organic matter retention was reduced in the disturbed sites. This constant residual level of detritus may be due to on-site production of algal detritus during spring and summer, coupled with some import of autumn leaf detritus from upstream reaches. Predisturbance measurements of benthic detritus were made in March 1983 by Speaker (1985), who reported a habitat-weighted average of 588 g/m^2 within the debris flow reach.

Leaf litter

Leaves in the wetted channel and on the active channel shelf represented current and potential sources of aquatic detritus for the different reaches (Fig. 4B). The wetted channel comprised 43–47% of the active channel in Quartz Creek when leaves were sampled in November 1987. In both zones, densities of leaves were 5–10 times higher in the upstream reach than in the debris flow or downstream reaches. In all three reaches, leaf densities were at least twice as high on the active channel shelf than in the wetted channel.

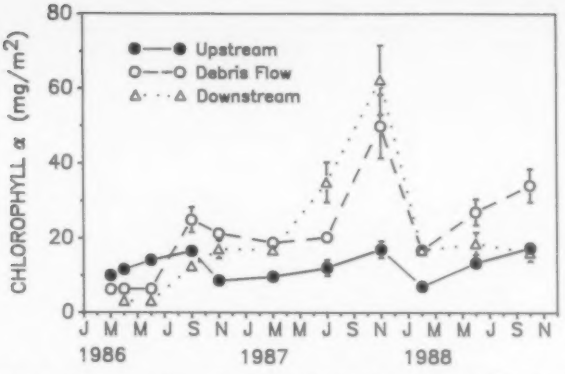


FIG. 6. Mean abundance (±SE) of algal chlorophyll *a* in the three study reaches of Quartz Creek from 1986 to 1988.

Benthic algae

Chlorophyll *a* abundance was used as an index of algal standing crop. During the 3-yr study, the amount of chlorophyll *a* was relatively constant in the upstream reach, varying from 8 to 16 mg/m^2 (Fig. 6). In the debris flow and downstream sites, chlorophyll *a* levels were low shortly after the disturbance but increased rapidly. By November 1986, mean chlorophyll *a* levels in the disturbed sites were two times higher than in the upstream site. These differences persisted into 1987, and by November 1987, mean chlorophyll *a* was about three times higher in the disturbed sites (50–60 mg/m^2) than in the upstream reach (15 mg/m^2). Late rains in 1987, coupled with relaxation of grazing pressure in the autumn, probably accounted for these high chlorophyll *a* levels. In 1988, differences in chlorophyll *a* diminished among sites, but the debris flow reach maintained the highest chlorophyll *a* levels. In all three reaches, chlorophyll *a* levels were lowest in winter.

Macroinvertebrates

One month after the flood and debris flow, macroinvertebrate densities were low at all sites, averaging <2000 animals/m^2 (Fig. 7A). Invertebrate abundance at the upstream site remained relatively constant at about 3000/m^2 through March 1987, but then increased to >10 000/m^2 in November 1987 before declining again in 1988. At the debris flow and downstream sites, invertebrate abundance fluctuated greatly, exhibiting summer – early autumn maxima and winter minima. In the summer of 1986 and from 1987 to 1988, mean invertebrate abundances at the disturbed sites were double those of the upstream site. In general, invertebrate densities at all sites were highest in the second year (1987) after the debris flow, reaching about 20 000/m^2 in the disturbed reaches and 12 000/m^2 at the upstream site.

Taxonomic richness of invertebrates at the upstream site was about 50 taxa shortly after the flood, and increased to 70–80 taxa during the following year (Fig. 7B). The number of taxa in the debris flow and downstream reaches was quite low shortly after the disturbance (25–35 taxa), but increased rapidly during the first summer. From November 1986 to June 1988, taxon richness was similar among the three reaches. Richness declined from November 1987 to June 1988 in all reaches, possibly associated with the effects of high flows that winter.

Fish populations

Trout populations were locally decimated by the 1986 debris flow. In October 1983, cutthroat trout density in the debris flow

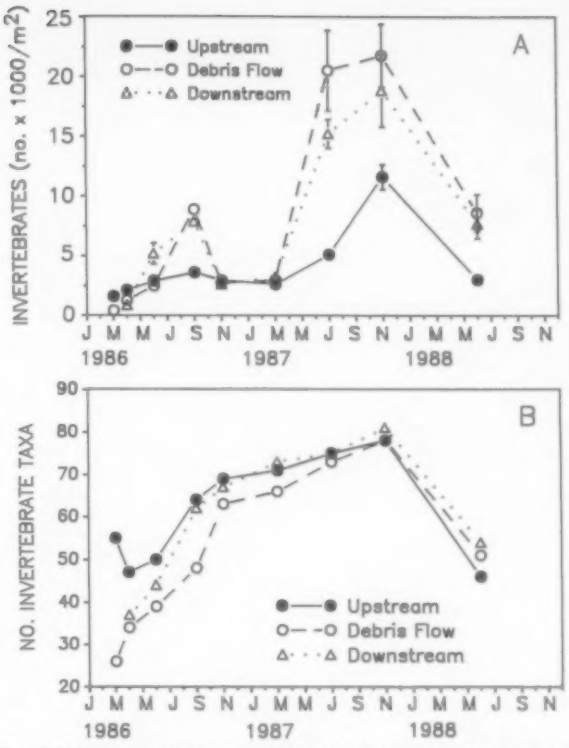


FIG. 7. Macroinvertebrate assemblages in the three study reaches of Quartz Creek from 1986 to 1988. (A) Mean density (±SE) of all macroinvertebrates; (B) total number of taxa found in each reach.

reach was 146 fish/100 m of stream length (Moore 1987). One month after the disturbance (March 1986), trout density in the debris flow reach was only about 10 fish/100 m and remained <20 fish/100 m throughout 1986 (Fig. 8A). Trout density in the upstream reach was about 100 fish/100 m in June 1986 and stayed near that level for the entire 3-yr study. Densities in the downstream reach were intermediate between the other two reaches. In 1987 and 1988, trout densities increased markedly in the debris flow and downstream reaches, and by October 1988, densities in the disturbed reaches were about double those of the upstream reach.

Adult cutthroat trout (1+ and older fish) and cutthroat fry (0+ fish or young-of-the-year) showed different responses to the debris flow. The upstream reach had the highest densities of adult trout in 1986 and 1987, averaging 50–70 fish/100 m (Fig. 8B). In contrast, densities of adult trout remained low in the debris flow site (<30/100 m) and intermediate in the downstream reach (40–50/100 m). In 1988, however, adult trout densities in the disturbed reaches increased sharply, especially in the downstream reach, and exceeded those of the upstream reach.

Cutthroat trout fry emerge from their gravel redds in May–June. Because some fry had not yet emerged at the time of the June sampling, the early autumn sample provided the best estimate of annual fry production (Fig. 9A). The upstream reach produced similar numbers of fry each year from 1986 to 1988 (30–50 fry/100 m), which is close to 1983 densities in the debris flow reach (61 fry/100 m). The debris flow reach produced very few fry in 1986 whereas normal densities were observed in the downstream reach (50 fry/100 m). In 1987 and 1988, the debris

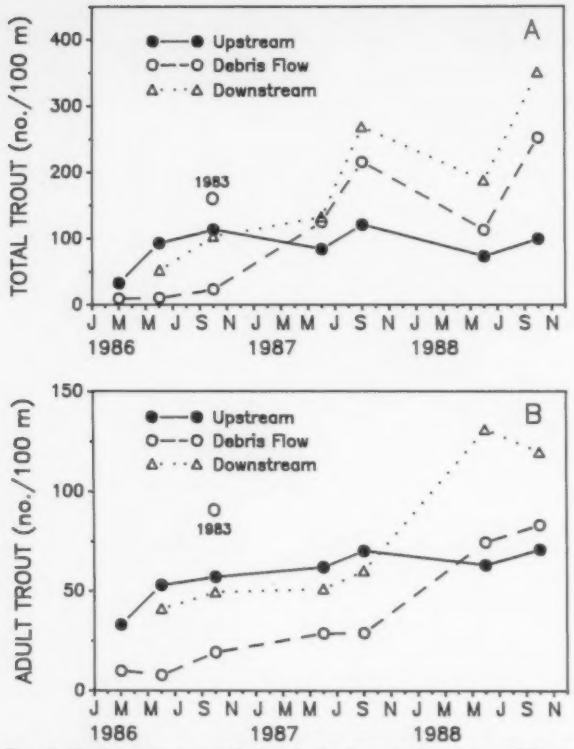


FIG. 8. Cutthroat trout populations in the three study reaches of Quartz Creek from 1986 to 1988. (A) Total trout abundance (fry plus adults); (B) abundance of adult trout only. Unconnected point refers to 1983 trout density in the debris flow reach.

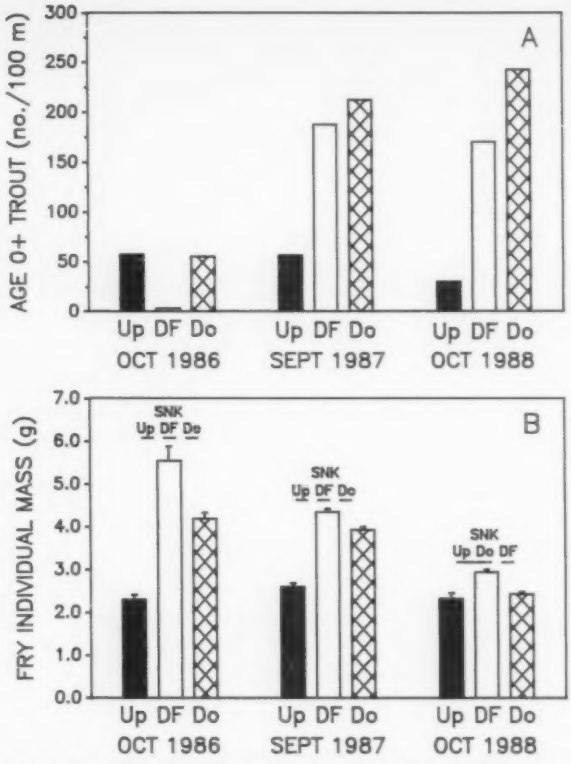


FIG. 9. Cutthroat fry populations in the three study reaches of Quartz Creek from 1986 to 1988. (A) Fry population density in the autumn of each year; (B) mean size (± SE) of individual fry, as wet mass, in the autumn of each year. SNK = results of a Student–Newmann–Keuls test where different underscores indicate significantly different mean masses ($p<0.05$).

flow and downstream reaches had high fry densities (approximately 200 fry/100 m), some four times greater than in the upstream reach.

Growth rates of fry also varied considerably among reaches (Fig. 9B). In the upstream reach, fry reached similar mass in the autumn of each year (approximately 2.5 g). In contrast, fry were 50–100% larger in the disturbed reaches during autumn of 1986 and 1987, indicating higher growth rates. However, this difference declined over time, and in 1988, fry size did not differ significantly between the upstream and downstream reaches, and fry were only about 10% larger in the debris flow reach.

Overwinter survival of fry was examined by comparing numbers of 0+ trout in the fall with numbers of 1+ trout the following spring (the same cohort) using size–frequency plots (summarized in Fig. 10). Numbers of 1+ trout in spring 1987 were 25–40% higher than 0+ trout densities in fall 1986 (except in the downstream reach), indicating immigration of fish into these reaches after the flood or debris flow. Lower than average rainfall in 1986–87 (resulting in low winter flows) probably also enhanced overwinter survival of fry. In 1987–88, cohort densities declined over the winter, but survival was twice as high in the upstream reach (only 35% of 0+ cohort lost) as in the debris flow reach (69% lost) or downstream reach (67% lost). High rainfall in 1987–88, and resultant high winter flows, probably contributed to low fry survival in the disturbed reaches.

Discussion

Value of Natural Experiments

Major disturbances such as the debris flow in Quartz Creek provide unique opportunities to assess ecosystem processes as part of "natural" experiments (Connell 1978; White and Pickett 1985). Such disturbances have realistic intensity and spatial extent, and ecological recovery patterns are a function of natural ecosystem resistance and resilience. However, three problems often hinder the evaluation of natural disturbance: (1) lack of predisturbance information or representative control systems, (2) limited knowledge of the timing, extent, and immediate effects of the event, and (3) inability to assess recovery processes over sufficiently broad spatial and temporal scales (Sousa 1984).

In Quartz Creek, these limitations were minimized by a combination of events. First, because the disturbance occurred in a previously studied stream reach, a substantial amount of predisturbance information was available. A suitable upstream control area also was located, which provided a parallel comparison over time with the debris flow disturbed areas. Second, we examined debris flow impact just 48 h after it occurred and were able to gauge its origin, mass, velocity, and distribution in the watershed. Immediate effects were assessed with qualitative observations, and quantitative sampling was initiated 1 mo after the event. Third, ecosystem recovery was

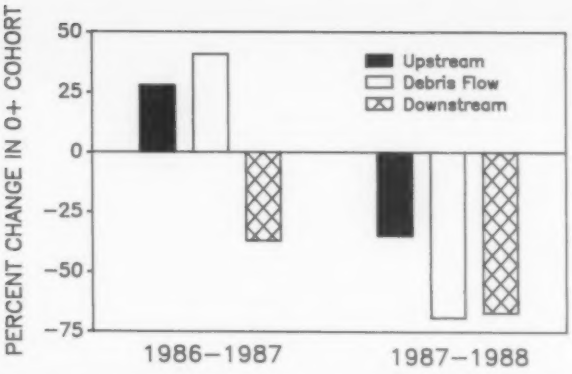


FIG. 10. Change in numbers of 0+ cohort of cutthroat trout between autumn and the following spring for 1986–87 and 1987–88 in the three study reaches of Quartz Creek. Positive change indicates net immigration whereas negative change indicates net mortality and emigration.

evaluated over broad spatial and temporal frameworks; we monitored over 600 m of Quartz Creek for 3 yr after the event. We will continue to monitor ecosystem recovery of Quartz Creek as part of the Long-Term Ecological Research Program at the H. J. Andrews Experimental Forest.

Stream Ecosystem Responses

Template for ecological recovery

The backdrop for physical and ecological recovery of Quartz Creek involved two spatial scales of disturbance and two levels of disturbance intensity. The flood in Quartz Creek occurred on a basin-wide scale but with only moderate intensity, as shown in the intact riparian vegetation and channel structure of the upstream reach. Refuge for organisms from the flood alone was available within lateral habitats and among coarse substrates. In contrast, the debris flow was intense but relatively localized, as shown in its dramatic impacts on a 500-m reach. In a confined area, riparian vegetation was destroyed and refuge for aquatic organisms was scarce or absent. Thus, two scales of disturbance mosaics emerged from the Quartz Creek flood and debris flow, each eliciting different recovery processes.

Geomorphic and hydraulic changes in Quartz Creek were obvious and immediate consequences of the debris flow. The stairstep sequence of pools and cascades typical of these montane streams changed to series of short, disordered units or longer, high-velocity units such as rapids and riffles. Sediment was eroded from the upper end of the debris flow reach and deposited at the lower end behind the debris accumulation, resulting in local reduction of stream gradient. During late summer and early autumn, streamflow went entirely subsurface through the deep sediments up to 50 m behind the debris dam. Surface flow reappeared only at the lower end of the debris dam.

Hydraulic retention in these streams normally is high because of good pool development, heterogeneous stream margins, and high channel roughness (Speaker 1985). Channel morphology was altered by the debris flow and hydraulics were simplified, resulting in rapid transport of water through the disturbed reach. Such declines in hydraulic retention can create a more rigorous environment for aquatic biota and may reduce nutrient retention (Lamberti et al. 1989). Thus, the debris flow created a channel of greatly modified geomorphic and hydraulic character.

The downstream reach also experienced a flood and mud flow, but much of the organic debris was deposited in the massive accumulation just upstream. Thus, the scouring and depositional action of the debris flow was diminished by the debris dam. It is likely that a significant proportion of the fauna and flora survived in this reach because of the lower intensity of the disturbance. The rapid biotic recovery of the downstream reach also may be related to the hydraulic "buffering" effect provided by the debris dam during subsequent high flow. However, the downstream reach may experience lingering detrimental effects of the debris flow because the debris dam may trap coarse sediments that would normally be delivered to the reach. The debris dam itself may variably act as either a source of organic matter for the downstream reach or as a sink for entrapment of detritus transported from upstream.

Basis of ecological recovery

Stimulation of primary production coupled with residual storage of detritus appeared to provide the trophic basis for ecological recovery in Quartz Creek. After September 1986, algal standing crop was consistently higher in the disturbed reaches. Such stimulation may have resulted from increased nutrient concentrations or light levels, or both. Disturbance in drainage basins often is associated with increases in dissolved nutrient concentrations (Likens et al. 1970). However, the debris flow disturbed only plant communities associated with the valley floor, and upslope nutrient cycling was not disrupted. As a result, we observed no significant increases in nitrogen or phosphorus concentrations in Quartz Creek.

Increased light levels are a more likely cause of enhanced primary production in Quartz Creek. Removal of streamside vegetation greatly increased irradiance levels in the debris flow and downstream reaches. Because benthic primary production in small forested streams is limited primarily by low light levels (Gregory 1980), opening of the canopy in Quartz Creek apparently stimulated algal production. This enhanced primary production occurred in spite of very low nutrient concentrations in the disturbed reaches.

The abundance of benthic detritus often is an important determinant of trophic structure in stream ecosystems (Vannote et al. 1980; Cummins et al. 1989). In Quartz Creek, the debris flow removed local riparian sources of leaf detritus (as shown in leaf densities), yet the disturbed reaches had moderate standing crops of benthic detritus. This may be because algae contributed to detritus pools in the disturbed reaches, or there was significant import of detritus from upstream reaches. Alternatively, this detritus may represent recalcitrant material in long-term storage within the sediments. Thus, increased primary production, coupled with a residual supply of detritus, probably formed the bioenergetic basis for stream ecosystem recovery from the debris flow.

Recovery of invertebrate assemblages

Macroinvertebrate populations in Quartz Creek recovered rapidly from the February 1986 disturbance. Within 6 mo, invertebrate densities in the debris flow impacted reaches exceeded those of the upstream reach. Within 9 mo, taxonomic diversity of the disturbed reaches equaled that of the upstream reach. However, in 1987, densities in all three reaches were higher than in 1986, probably indicating that recovery processes from the large flood were still ongoing. Apparently, some invertebrate populations recovered from the localized debris flow within 1 yr whereas more time was needed for com-

plete recovery of the invertebrate assemblage from the basin-wide flood.

We propose that increased invertebrate densities in the disturbed reaches were a direct response to increased primary production. In 1986, invertebrate assemblages in the debris flow and downstream reaches were composed mostly of herbivorous taxa. Chironomid midge larvae and baetid mayflies, which tend to be early colonists of disturbed stream habitats (Lamberti and Moore 1984), were particularly abundant in the impacted reaches. This rapid recolonization probably occurred primarily by drift of larvae from upstream areas and oviposition by adults. In 1987, less mobile grazers such as glossosomatid caddisflies and pleurocerid snails, and various detritivores and predators, were found in low densities within the disturbed reaches. This longer term recolonization probably occurred by a combination of drift, oviposition, and benthic movement (Williams and Hynes 1976), which brought less vagile taxa into the disturbed reaches. In Quartz Creek, the highest chlorophyll *a* levels and the highest invertebrate densities were both observed in late 1987, suggesting a tight linkage between primary and secondary production. Previous studies in streams of the Pacific Northwest also have reported higher macroinvertebrate densities and algal biomass in reaches with open canopies as compared with shaded reaches (Murphy et al. 1981; Hawkins et al. 1982).

Recovery of fish populations

Two years after the debris flow, trout populations in the disturbed reaches had recovered to levels equal to or exceeding those in the upstream reach. Fish population recovery appeared to be related to (1) immigration of 1+ trout during the first year after the disturbance and (2) enhanced recruitment of fry in the second and third years. Fry were virtually eliminated in the debris flow reach during 1986 but were abundant in 1978 and 1988, thus contributing to large 1+ cohorts in each succeeding year in spite of poor overwinter survival. Thus, high fry recruitment in summer apparently compensated for increased winter losses.

Low fry density in the debris flow reach during 1986 was probably related to timing of the debris flow and the life history and behavior of cutthroat trout. In Cascade Mountain streams, age 2+ and older trout typically spawn from February to April and fry emerge from redds about 8 wk later (Behnke 1979; Moore and Gregory 1988a). Any redds built prior to the February 24 debris flow undoubtedly were destroyed by the event, as were most fish of reproductive age. Thus, in 1986 there was little reproduction within the debris flow reach and few fry migrated into the reach, as cutthroat fry tend to remain close to their spawning areas (Moore and Gregory 1988b). Other studies also have shown that eggs and juveniles of salmonids are affected more strongly by lotic disturbance than are adults (Hanson and Waters 1974; Seegrist and Gard 1972).

In the downstream reach, populations of both fry and adults recovered more rapidly than in the debris flow reach. Both fish and some redds probably survived downstream because of lighter disturbance, and more reproductive adults were present in 1986 to contribute to first-year fry recruitment. Unlike the debris flow reach, upstream movement of adult fish into this reach was unimpeded by a debris dam.

In 1986 and 1987, trout fry grew to larger size in the open disturbed reaches than in the shaded upstream reach. This pattern may be related to (1) food abundance, (2) food capture rates, and (3) total fish abundance in the disturbed reaches. Macroinvertebrates were more abundant in the disturbed reaches, especially drifting forms such as mayflies and midges that are important food items for cutthroat trout (Moore and Gregory 1988a). Prey capture efficiencies also were potentially higher in the disturbed reaches because of increased light levels (Wilzbach et al. 1986). Thus, a combination of abundant food and efficient capture probably contributed to short-term enhanced growth of fry in the disturbed reaches (see also Murphy and Hall 1981). However, fry growth rates in the disturbed reaches declined from 1986 to 1988 as trout densities steadily increased. Over time the advantages of increased food production appeared to be balanced by higher fish densities in the disturbed reaches. Competition among fish for food or space, coupled with declining invertebrate abundances in 1988, may have resulted in density-dependent limitation of fry growth in Quartz Creek.

Disturbance in Stream Ecosystems

Most stream ecosystems are subjected to disturbance by floods that occur anywhere from several times per year (Fisher 1983) to several times per decade (Harr 1981). In montane streams of the Pacific Northwest, floods occur once every 1–2 yr on average, normally in the late autumn through early spring. Compared with other major disturbances such as wildfire or hurricanes (Connell 1978), flood disturbance in streams is frequent and relatively predictable. Stream organisms are adapted to this flood disturbance regime and have evolved various behavioral or reproductive strategies to minimize death or displacement or to ensure population recovery (e.g. Siegfried and Knight 1977; Fisher et al. 1982; Meffe 1984). In contrast, debris flows are rare, episodic events that may influence a particular stream reach only once every 50–200 yr or even longer (Swanson et al. 1987). These events have varying areal extent, high intensity, and low predictability. Loss of organisms during debris flows appears to be severe.

Sousa (1984) proposed that ecosystem recovery following disturbance is a function of (1) resistance of extant species to the disturbance, (2) influx rates of new organisms, (3) characteristics of the disturbance, including severity and time of occurrence relative to organism life history, and (4) features of the disturbed patch, including size, location, and internal heterogeneity. In Quartz Creek, we presume that the debris flow overwhelmed the resistance of most organisms in directly affected areas, as seen in the abrupt and severe reduction in plant and animal abundance. Colonization of the disturbed area thus was primarily a function of immigration rates of nearby organisms.

The rate of biotic recovery varied with the trophic level, proximity, and dispersal ability of colonists. Microbial and algal propagules are nearly always present in stream water (Lamberti and Resh 1987), and their downstream transport provides a continual source of colonists. Algal immigration and colonization provided the propagules for algal growth, and the enhanced light levels of the disturbed reaches favored rapid recovery of algal standing crop in Quartz Creek.

Recovery of consumer populations was slower than for algae, but still relatively rapid. Initial (first-year) recolonization by macroinvertebrates consisted primarily of vagile herbivores, but by the second year a fuller complement of trophic groups had recolonized the disturbed areas. This rapid recolonization may have been related to (1) physical stabilization of the channel, (2) increased food availability, such as periphyton, and (3)

recovery of individual populations following recolonization. For invertebrates, the lower intensity but more extensive effects of the flood may have been more important to recolonization dynamics than the higher intensity but less extensive debris flow.

Among the biota, recovery of fish populations was the most gradual and occurred both by immigration into the disturbed reaches and reproduction within the reach. Fry grew rapidly in the disturbed reaches during benign summer conditions, but about two thirds were eliminated by rigorous winter conditions and possibly predation. Winter fry loss in the disturbed reaches probably was related to the more rigorous hydraulic conditions and reduced overwintering habitat in the affected areas. During high flow, availability of lateral habitat as refuge is critical to the survival of cutthroat trout fry (Moore and Gregory 1988b).

Although the debris flow in Quartz Creek had severe local effects, the size of the disturbance (approximately 0.5 km of mainstem length) was small compared with some debris flows that may move for several kilometres or more in stream channels (Swanson et al. 1987). Its occurrence in February was prior to the major spring dispersal period for aquatic invertebrates (Williams and Hynes 1976) and cutthroat trout (Wyatt 1959). Disturbance size and timing thus favored rapid recolonization of the affected reaches. In general, this rapid recolonization may reflect some preadaptation to episodic disturbance imparted by adaptation to physically similar but more regular and frequent disturbance (i.e. floods).

Concluding Remarks

Stream ecosystem properties are closely linked to the structure of riparian vegetation and to the valley floor processes influencing plant succession (Hynes 1975; Vannote et al. 1980; Minshall et al. 1983; Gregory et al. 1991). Valley floors are dynamic portions of the landscape that are frequently affected by fluvial and terrestrial disturbance. Disturbance modifies valley floors through physical rearrangement of geomorphic surfaces, destruction of riparian vegetation, and direct removal of stream biota. The Quartz Creek study suggests that recovery of stream ecosystems from major disturbance is a complex process linked to postdisturbance stabilization of geomorphic surfaces, regrowth of riparian vegetation, development of heterogeneous hydraulic environments, and recolonization potential of aquatic organisms.

There is growing recognition that long-term studies are needed to examine complex ecological processes (Schlosser 1982; Callahan 1984). This 3-yr study of Quartz Creek documents the early phases of ecological recovery from a severe disturbance. Organismal abundances and ecological processes in the disturbed segment of Quartz Creek continue to fluctuate broadly, an indication that ecosystem stability (sensu Connell and Sousa 1983) has not yet been achieved. However, overall rates of recolonization of the disturbed areas were rapid, indicating high resilience in lotic biota that reflects ecological responses shaped by the frequent disturbance in stream ecosystems. Long-term monitoring of ecosystem properties in disturbed systems such as Quartz Creek should contribute substantially to our understanding of disturbance ecology.

Acknowledgments

We thank Frederick Swanson, Gordon Grant, and Arthur McKee for providing geomorphic data and for valuable discussion. Comments by Peter Bisson, Stuart Fisher, and Colin Levings improved an earlier version of the manuscript. Chemical analyses were performed by Cameron Jones in the Cooperative Chemistry Analytical Laboratory of the USFS-PNW Research Station and Oregon State University. This research was supported by research grant BSR-8508356 from the National Science Foundation and by the Long-Term Ecological Research Program of NSF (BSR-8514325).

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Armour, C. L., K. P. Burnham, and W. S. Platts. 1983. Field methods and statistical analyses for monitoring small salmonid streams. U.S. Fish Wildl. Serv. FWS/OBS-83/33: 200 p.

Behnke, R. J. 1979. Monograph of the native trouts of the genus *Salmo* of western North America. U.S. Forest Service, Washington, DC. 163 p.

Callahan, J. T. 1984. Long-term ecological research. BioScience 34: 363–367.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science (Wash., DC) 199: 1302–1310.

Connell, J. H., and W. P. Sousa. 1983. On the evidence needed to judge ecological stability or persistence. Am. Nat. 121: 787–824.

Cummins, K. W., M. A. Wilzbach, D. M. Gates, J. B. Perry, and W. B. Taliaferro. 1989. Shredders and riparian vegetation. BioScience 39: 24–30.

Fisher, S. G. 1983. Succession in streams, p. 7–27. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology. Plenum Press, New York, NY.

Fisher, S. G., L. J. Gray, N. B. Grimm, and D. E. Busch. 1982. Temporal succession in a desert stream ecosystem following flash flooding. Ecol. Monogr. 52: 93–110.

Friday, J., and S. J. Miller. 1984. Statistical summaries of streamflow data in Oregon. Vol. 2. Western Oregon. U.S. Geol. Surv. Open-File Rep. 84-454, Portland, OR. 250 p.

Grant, G. E. 1986. Downstream effects of timber harvest activities on the channel and valley floor morphology of western Cascade streams. Ph.D. thesis, Johns Hopkins University, Baltimore, MD. 349 p.

Gregory, S. V. 1980. Effects of light, nutrients, and grazing on periphyton communities in streams. Ph.D. thesis, Oregon State University, Corvallis, OR. 151 p.

Gregory, S. V., F. J. Swanson, W. A. McKee, and K. W. Cummins. 1991. An ecosystem perspective of riparian zones. BioScience. (In press)

Hanson, D. L., and T. F. Waters. 1974. Recovery of standing crop and production rate of a brook trout population in a flood-damaged stream. Trans. Am. Fish. Soc. 103: 431–439.

Harr, R. D. 1981. Some characteristics and consequences of snowmelt during rainfall in western Oregon. J. Hydrol. 53: 277–304.

Hawkins, C.P., M. L. Murphy, and N. H. Anderson. 1982. Effects of canopy, substrate composition, and gradient on the structure of macroinvertebrate communities in Cascade Range streams of Oregon. Ecology 63: 1840–1856.

Hynes, H. B. N. 1975. The stream and its valley. Verh. Int. Ver. Limnol. 19: 1–15.

Krebs, C. J. 1989. Ecological methodology. Harper and Row, New York, NY. 654 p.

Lamberti, G. A., S. V. Gregory, L. R. Ashkenas, R. C. Wildman, and A. D. Steinman. 1989. Influence of channel geomorphology on retention of dissolved and particulate matter in a Cascade Mountain stream, p. 33–39. *In* D. L. Abell [ed.] Proc. Calif. Riparian Syst. Conf., Gen. Tech. Rep. PSW-110. Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, Berkeley, CA.

Lamberti, G. A., and J. W. Moore. 1984. Aquatic insects as primary consumers, p. 164–195. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, New York, NY.

Lamberti, G. A., and V. H. Resh. 1987. Seasonal patterns of suspended bacteria and algae in two northern California streams. Arch. Hydrobiol. 110: 45–57.

Likens, G. E., F. H. Bormann, N. M. Johnson, D. W. Fisher, and R. S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40: 23–47.

McElravy, E. P., G. A. Lamberti, and V. H. Resh. 1989. Year-to-year variation in the aquatic macroinvertebrate fauna of a northern California stream. J. North Am. Benthol. Soc. 8: 51–63.

MEFFE, G. K. 1984. Effects of abiotic disturbance on coexistence of predator–prey fish species. Ecology 65: 1525–1534.

MINSHALL, G. W., R. C. PETERSON, K. W. CUMMINS, T. L. BOTT, J. R. SEDELL, C. E. CUSHING, AND R. L. VANNOTE. 1983. Interbiome comparison of stream ecosystem dynamics. Ecol. Monogr. 53: 1–25.

MOORE, K. M. S. 1987. Ecology of aquatic habitats associated with stream margins. M.S. thesis, Oregon State University, Corvallis, OR. 130 p.

MOORE, K. M. S., AND S. V. GREGORY. 1988a. Summer habitat utilization and ecology of cutthroat trout fry (*Salmo clarki*) in Cascade Mountain streams. Can. J. Fish. Aquat. Sci. 45: 1921–1930.

1988b. Response of young-of-the-year cutthroat trout to manipulation of habitat structure in a small stream. Trans. Am. Fish. Soc. 117: 162–170.

MURPHY, M. L., AND J. D. HALL. 1981. Varied effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. Can. J. Fish. Aquat. Sci. 38: 137–145.

MURPHY, M. L., C. P. HAWKINS, AND N. H. ANDERSON. 1981. Effects of canopy modification and accumulated sediment on stream communities. Trans. Am. Fish. Soc. 110: 469–478.

PICKETT, S. T. A., AND P. S. WHITE. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, London. 472 p.

REPLOGLE, J. A., L. W. MYERS, AND K. J. BURST. 1976. Flow measurement with fluorescent tracers. J. Hydraul. Div. ASCE No. HY5: 1–14.

RESH, V. H., A. V. BROWN, A. P. COVICH, M. E. GURTZ, H. W. LI, G. W. MINSHALL, S. R. REICE, A. L. SHELDON, J. B. WALLACE, AND R. C. WISSMAR. 1988. The role of disturbance in stream ecology. J. North Am. Benthol. Soc. 7: 433–455.

SCHLOSSER, I. J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecol. Monogr. 52: 395–414.

SEEGRIST, D. W., AND R. GARD. 1972. Effects of floods on trout in Sagehen Creek, California. Trans. Am. Fish. Soc. 101: 478–482.

SIEGFRIED, C. A., AND A. W. KNIGHT. 1977. The effects of washout in a Sierra foothill stream. Am. Midl. Nat. 98: 200–207.

SOUSA, W. P. 1984. The role of disturbance in natural communities. Annu. Rev. Ecol. Syst. 15: 353–391.

SPEAKER, R. W. 1985. Distribution and retention of particulate organic matter in streams of the Cascade Mountains, Oregon. M.S. thesis, Oregon State University, Corvallis, OR. 145 p.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 311 p.

SULLIVAN, K., T. E. LISLE, C. A. DOLLOFF, G. E. GRANT, AND L. M. REID. 1987. Stream channels: the link between forests and fishes, p. 39–97. *In* E. O. Salo and T. W. Cundy [ed.] Streamside management: forestry and fishery interactions. Institute of Forest Resources, University of Washington, Seattle, WA.

SWANSON, F. J., L. E. BENDA, S. H. DUNCAN, G. E. GRANT, W. F. MEGAHAN, L. M. REID, AND R. R. ZIEMER. 1987. Mass failures and other processes of sediment production in Pacific Northwest forest landscapes, p. 9–38. *In* E. O. Salo and T. W. Cundy [ed.] Streamside management: forestry and fishery interactions. Institute of Forest Resources, University of Washington, Seattle, WA.

SWANSON, F. J., AND G. W. LIENKAEMPER. 1978. The physical consequences of large organic debris in Pacific Northwest streams. USDA For. Serv. Gen. Tech. Rep. PNW-16: 12 p.

SWANSTON, D. N., AND F. J. SWANSON. 1976. Timber harvesting, mass erosion, and steepland forest geomorphology in the Pacific Northwest, p. 199–221. *In* D. R. Coates [ed.] Geomorphology and engineering. Dowden, Hutchinson, and Ross, Stroudsburg, PA.

VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

WHITE, P. S., AND S. T. A. PICKETT. 1985. Natural disturbance and patch dynamics: an introduction, p. 3–13. *In* S. T. A. Pickett and P. S. White [ed.] The ecology of natural disturbance and patch dynamics. Academic Press, London.

WILLIAMS, D. D., AND H. B. N. HYNES. 1976. The recolonization mechanisms of stream benthos. Oikos 27: 265–272.

WILZBACH, M. A., K. W. CUMMINS, AND J. D. HALL. 1986. Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. Ecology 67: 898–911.

WYATT, B. 1959. Observations on the movements and reproduction of the Cascade form of cutthroat trout. M.S. thesis, Oregon State University, Corvallis, OR. 60 p.

Salinity Tolerance and Osmoregulation in the Nemertean *Pseudocarcinonemertes homari*, an Egg Predator of American Lobster, *Homarus americanus*

G. Charmantier and M. Charmantier-Daures

Laboratoire d'Ecophysiologie des Invertébrés, Université des Sciences et Techniques du Languedoc, 34095 Montpellier Cedex 05, France

and S. L. Waddy and D. E. Aiken

Department of Fisheries and Oceans, Aquaculture and Invertebrates Fisheries, Biological Station, St. Andrews, N.B. E0G 2X0, Canada

Charmantier, G., M. Charmantier-Daures, S. L. Waddy, and D. E. Aiken. 1991. Salinity tolerance and osmoregulation in the nemertean *Pseudocarcinonemertes homari*, an egg predator of American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 48: 209–214.

The salinity tolerance and osmoregulation of *Pseudocarcinonemertes homari* were determined to develop a method for elimination of this nemertean from egg masses of American lobster, *Homarus americanus*. The lower and upper lethal salinities (LS50) for nemerteans are 11 and 45‰ at 7°C and 19 and 37‰ at 14.5°C. Immersion in freshwater was lethal in 4 min or less, depending on temperature. The nemerteans hyperosmoconformed over the range 10–1600 mosm·kg^{-1}. Lobster eggs (eye index ≤500 μm) survived for 2–8 h when immersed in freshwater. Ovigerous lobster were not affected by a 5-min immersion in freshwater and were able to withstand at least 30 min if only the abdomen was immersed. Lobster eggs hatched and larvae developed normally up to at least stage IV, even after a 30-min freshwater immersion. These results suggest that nemerteans could be eliminated from American lobster by immersing their abdomen and attached eggs in freshwater for 5 min and immersing the entire lobster for an additional 5 min.

La tolérance à la salinité et l'osmorégulation de *Pseudocarcinonemertes homari* ont été étudiées afin d'établir une méthode d'élimination de ce némerte des masses d'oeufs du homard Américain, *Homarus americanus*. Les salinités léthales inférieures et supérieures pour le némerte (LS 50) sont de 11 et 45‰ à 7°C et 19 et 37‰ à 14,5°C. L'immersion en eau douce est léthale en 4 min ou moins selon la température. Ce némerte est hyperosmoconformeur pour la gamme de salinité 10–1 600 mosm·kg^{-1}. Les oeufs de homard (index d'oeil ≤ 500 μm) survivent de 2 à 8 h à une immersion en eau douce. Des femelles ovigères de homard peuvent supporter sans dommage une immersion totale de 5 min en eau douce, ou une immersion de l'abdomen seul de 30 min au moins. Après une immersion de 30 min en eau douce, les oeufs de homard éclosent normalement et les larves se développent normalement jusqu'au stade IV. Ces résultats montrent que les némertes présents sur des homards Américains pourraient été éliminés par une immersion de 5 min en eau douce de l'abdomen et des oeufs, suivie par une immersion totale de 5 min du homard.

Received April 9, 1990
Accepted August 10, 1990
(JA540)

Reçu le 9 avril 1990
Accepté le 10 août 1990

The nemertean *Pseudocarcinonemertes homari* feeds on American lobster, *Homarus americanus*, egg yolk, and infestation usually results in total destruction of the lobster egg mass (Aiken et al. 1985; Waddy and Aiken 1985). Despite the potential impact of *P. homari* and other nemertean egg predators on crustacean egg production (Campbell and Brattey 1986; Kuris and Wickham 1987), little is known about the physiology of *P. homari* or that of the various species of *Carcinonemertes*. The only data on salinity tolerance and volume regulation (linked to osmotic regulation) of nemerteans are from the marine free-living species *Lineus viridis*, *L. ruber*, *Procephalothrix spiralis*, and *Paranemertes peregrina* (Lechenault 1965a, 1965b; Ling 1969, 1970, 1971; Ferraris and Schmidt-Nielsen 1982; Amerongen and Chia 1983; Ferraris 1984, 1985a; Ferraris and Norenburg 1988).

The objective of this study was to determine the salinity tolerance and type of osmoregulation in *P. homari*. This would not only add to our knowledge of nemertean physiology, but by comparing the salinity tolerance of *P. homari* and lobster eggs, it was hoped that a technique could be developed for eliminating the nemerteans that would not harm the maternal female and attached eggs.

Materials and Methods

Animals

Nemerteans were collected in May 1988 from an ovigerous female *H. gammarus* crossed with a male American lobster. This lobster had been held in the lobster culture facility of the Biological Station, St. Andrews, N.B., since 1982 and had been infested with nemerteans several times. American lobsters used in survival and osmoregulation experiments were collected in the Bay of Fundy and Northumberland Strait.

Preparation of Media and Measurement of Osmotic Pressure

Dilute media were prepared by the addition of tap water to seawater, and high-salinity media were prepared by adding

Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) to seawater. Experiments on nemerteans were conducted at 7°C (local seawater temperature in late May) and/or 14.5°C. Salinities were expressed as osmotic pressure (milliosmoles per kilogram) and salt content (parts per thousand). Osmotic pressure was measured on an Advanced Instruments 31 LA osmometer and salinity on an YSI 33 salinometer. A value of 3.4‰ was equivalent to 100 mosm·kg^{-1}.

Survival Bioassays

To determine the salinity tolerance of nemerteans, acute static bioassays were conducted in test media differing by increments of 100 mosm·kg^{-1} and ranging from freshwater (≈10 mosm·kg^{-1}) through seawater (≈900 mosm·kg^{-1}) to high-salinity media (1600 mosm·kg^{-1}). During bioassays, groups of 10 nemerteans ranging in size from 0.8 to 8.0 mm were held communally in plastic weight boats in 200 mL of medium. Each test was replicated. After 0.5, 1, 2, 3, 6, 12, 24, 48, and 96 h, dead nemerteans were removed and counted (according to Sprague (1969) for toxicity studies). Criteria for determining death were lack of movement and response after repeated probing. Median times to 50% mortality (LT50) and 95% confidence limits were determined from a computer program based on probit analysis (Lieberman 1983). Median lethal salinities (LS50) and 95% confidence intervals were calculated using the Letcur program (Zitko 1982).

To more precisely evaluate the tolerance of nemerteans to freshwater, their LT50 was determined. Groups of 10 nemerteans were immersed in freshwater for 1, 2, 3, 4, 5, 10, 20, or 30 min and were then transferred back to seawater. The number of dead animals was determined 5 min and 1, 2, 3, 6, 24, 48, 72, and 96 h after their return to seawater. Nemerteans judged to be dead were retained in seawater in individual vials until the conclusion of the experiment to determine if they recovered. Each experiment was replicated and run at both 7 and 14.5°C.

Tolerance of lobster embryos to freshwater was determined from eggs collected from wild ovigerous females at different times of the year. The stage of development of the embryos was determined from the eye index (Perkins 1972). The LT50 was determined by immersing groups of 10 eggs in freshwater (12–14.5°C). After 0.25, 0.5, 1, 2, 3, 6, and 24 h, the number of dead embryos (cessation of heart beat) was determined. Each test was replicated.

To determine tolerance to freshwater, 27 adult intermolt lobsters weighing 300–500 g were partially (abdomen immersed, cephalothorax held above the water surface) or totally immersed in 20 L of freshwater at 10–12°C. In ovigerous females, the eye index of the embryos was determined prior to treatment and females were retained until their eggs hatched. Stage I lobster larvae were cultured to stage IV in 40-L planktonkreisels (Hughes et al. 1974) in flow-through seawater at 20°C and were fed frozen adult *Artemia*.

Osmoregulation

Nemerteans were held in groups of 10–15 in 200-mL weigh boats at 14.5°C at salinities ranging from 10–1600 mosm·kg^{-1}. After exposure, the nemerteans were partially dried on filter paper and then immersed in mineral oil to avoid evaporation and desiccation. The medium remaining on the nemerteans was aspirated with a micropipette. The extracellular fluid (ECF) was sampled by inserting a clean micropipette into the body of the nemertean. Osmotic pressure of the ECF was measured with reference to the osmotic pressure of the medium on a Kalber–Clifton microosmometer (Frick and Sauer 1973). Due to the opacity of the ECF, the visibility of ice crystals had to be improved by placing polarizing filters in the light path, as done with fish egg fluid (Holleland and Fyhn 1986).

After immersion in freshwater, intermolt lobsters weighing 300–500 g were kept in running seawater at seasonal temperature (7–9°C) and were fed fish and shrimp. Seven and 25 d after exposure to freshwater, lobsters were transferred to a 200-L tank and the salinity was progressively lowered from ≈900 mosm·kg^{-1} (31‰) to 500 mosm·kg^{-1} (17‰) by addition of tap water. Salinity decreased about 100 mosm·kg^{-1} every 10 h. A final salinity of 17‰ was chosen because previous experiments (Charmantier et al. 1984) demonstrated that in media of 500 mosm·kg^{-1} (17‰ salinity) the survival rate of lobsters is about 100%. The difference between the osmotic pressure of the hemolymph and the medium, reflecting ability to hyperregulate, was large enough (usually >100 mosm·kg^{-1}) to reflect interference on osmotic regulation from either internal or external factors. Therefore, 17‰ salinity was used for the osmoregulation study.

The recirculating water was aerated and filtered through polyurethane foam and activated charcoal. Temperature was regulated to 9°C and lobsters were not fed during the duration of the experiment. Titrations of hemolymph osmotic pressure were done after the lobsters had spent 4 d in the 500 mosm·kg^{-1} medium which allowed equilibration of the hemolymph osmotic pressure. Hemolymph was collected by inserting a hypodermic needle into the hemocoel through the sternal membrane of the second abdominal segment.

Results

Salinity Tolerance of *P. homari*

Pseudocarcinonemertes homari proved to be a euryhaline nemertean (Fig. 1). At 7°C, their LS50 in low-salinity media was ≈260 mosm·kg^{-1} (≈9‰) after 24 h and ≈320 mosm·kg^{-1} (11‰) (range 210–500 mosm·kg^{-1}, 7–17‰) after 96 h. In concentrated media, the corresponding LS50s were ≈ 1480 and 1350 mosm·kg^{-1} (≈50 and 45‰).

Elevated temperature reduced salinity tolerance; there were highly significant differences ($p < 0.001$) between 96-h LS50s at 7 and 14.5°C. After 96 h at 14.5°C, low and high LS50s were ≈560 and 1080 mosm·kg^{-1} (≈19 and 37‰); corresponding values at 7°C were ≈320 and 1350 mosm·kg^{-1} (≈11 and 45‰). No appreciable differences were detected between the results obtained from males and females, or from animals of different sizes.

Tolerance of *P. homari* to Freshwater

Median lethal times (LT50) in freshwater were 2.2 min at 7°C (95% confidence interval 1.8–2.7 min) and 1.1 min at 14.5°C (95% confidence interval 0.8–1.3 min). The minimum freshwater immersion that caused 100% mortality in the subsequent 48 or 96 h was 4 min at 7°C and 2 min at 14.5°C. All the nemerteans died within 1 h after a 5-min immersion in freshwater and within 5 min after a 10-min immersion in freshwater. Results were similar whether the nemerteans were isolated from or adherent on lobster eggs.

The nemerteans reacted to freshwater with violent movements that lasted about 1.5 min. After 3 min in freshwater, the nemerteans became immobile. During freshwater immersion,

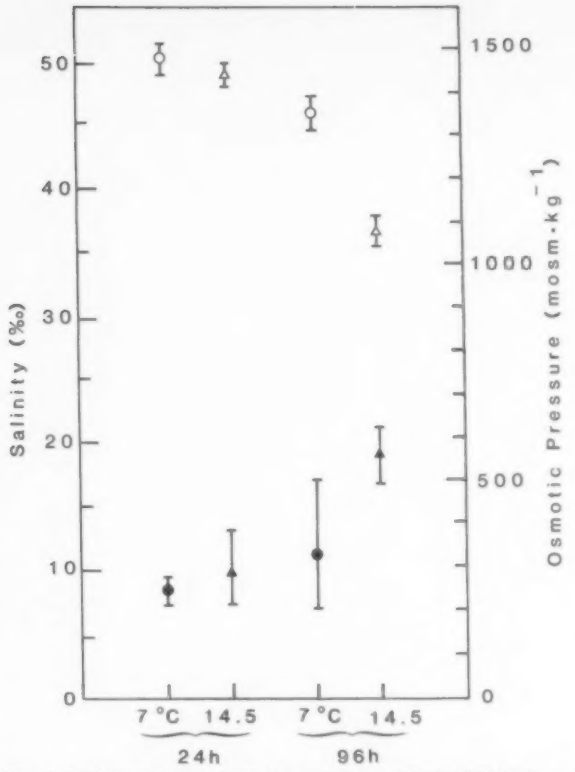


FIG. 1. Salinity tolerance in *P. homari*: 24- and 96-h LS50 in ‰ and mosm·kg^{-1} at 7°C (circles) and 14.5°C (triangles) in low-salinity (solid symbols) and high-salinity (open symbols) media. Each point represents the mean value of two determinations from 10 animals (95% confidence intervals indicated).

the volume of the nemerteans increased, a mucus-like substance surrounded the worms, adhesion to the eggs decreased, and the nemerteans became detached if the surrounding water was agitated.

Osmoregulation of *P. homari*

Adaptation time

After transfer from seawater (890 mosm·kg^{-1}, 30.3‰) to a dilute medium (500 mosm·kg^{-1}, 17‰), the osmotic pressure of the extracellular fluid stabilized within 0.5 h (Fig. 2). In subsequent osmoregulation experiments, the animals were held for 24 h in each medium before sampling.

Osmotic regulation

The nemertean hyperosmoconformed over the range of salinities tested. The osmotic pressures of ECF and the rearing medium differed by about 15–35 mosm·kg^{-1} (Fig. 3). The osmotic pressure of ECF did not vary significantly with salinity, except in the 300 mosm·kg^{-1} medium where it was significantly lower (*t*-test: $p < 0.05$).

Tolerance of Lobster and their Embryos to Freshwater

Tolerance of adult lobster to freshwater

Immersion of the lobster abdomen in freshwater for up to 30 min did not cause mortality (Table 1). Immersion of the entire lobster for 5 min in freshwater, followed or not by

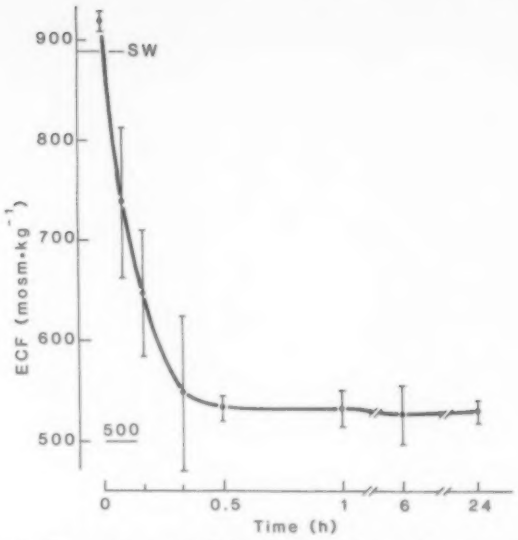


FIG. 2. Change in extracellular fluid (ECF) osmotic pressure in *P. homari* after rapid transfer from seawater (890 mosm·kg^{-1}, 30.3‰) to a dilute medium (500 mosm·kg^{-1}, 17‰) at 14.5°C. Each point represents the mean value of determinations from 5–10 animals (95% confidence intervals indicated).

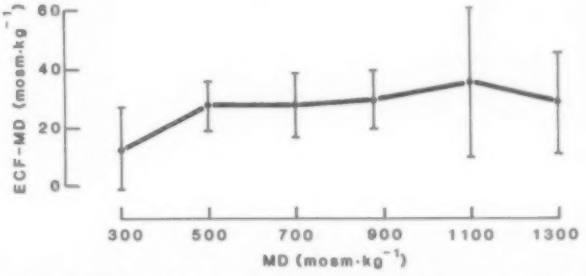


FIG. 3. Differences between the osmotic pressures of extracellular fluid and rearing medium (ECF − MD) according to the osmotic pressure of the rearing medium (MD) in *P. homari* at 14.5°C. Each point represents the mean value of determinations from 10 animals (95% confidence intervals indicated).

abdominal immersion, was tolerated; all of the 10 experimental lobsters survived and no adverse effects were seen over the next 2 mo. Immersions of 10–15 min in freshwater produced mortality; death occurred 6–24 h after return to seawater and there were signs of osmotic shock (swollen membranes and extended appendages). Given the relatively small number of tested animals, no differences were detected between males and females and between ovigerous and barren females.

Tolerance of lobster embryos to freshwater

Lobster embryos can survive for several hours in freshwater. Their LT50s range from 6 to 8 h when the eye indices are greater than 300 μm (Table 2). The tolerance of lobster eggs to freshwater is somewhat lower at the beginning of development and just before hatching (eye index of about 560 μm); however, even at these stages the LT50s are still greater than 2 h.

The long-term effect of freshwater immersions on embryonic and larval development was also determined. Egg masses carried by ovigerous females were immersed in freshwater, either

TABLE 1. Tolerance of adult *H. americanus* to freshwater. Number of lobster deaths following total and/or abdominal immersion.

Duration of total immersion (min)	Duration of abdominal immersion (min)	Number and sex of lobsters ♀	♂	Lobster deaths
0	15	2	—	0
0	30	2	—	0
5	0	4	1	0
5	5	5	—	0
10	0	4	1	0
10	5	2	—	1
15	0	4	2	2

TABLE 2. Tolerance of *H. americanus* embryos to freshwater. Median times and 95% confidence intervals to 50% mortality (LT50) at 12–14.5°C; each LT50 is the mean value of two determinations ($n = 20$).

Lobster egg eye index (μm)	LT50 (h)
197 ± 10	3.7 (3.0–4.4)
328 ± 5	6.7 (5.5–8.9)
376 ± 17	8.7 (7.2–13.6)
450 ± 18	7.9 (6.1–10.1)
554 ± 8	2.9 (2.4–3.4)

just the abdomen or the entire female. Freshwater immersions were performed for different durations at different stages of embryonic development: 10 min (eye indices 240 ± 7, 440 ± 14, 503 ± 12, 520 ± 11, and 537 ± 9 μm, 15 min (290 ± 15, 380 ± 6, and 440 ± 10 μm), and 30 min (316 ± 19 and 475 ± 11 μm). They usually did not result in any detectable damage to the egg membranes, embryos, or larvae; neither egg loss nor embryo mortality was detected. Time required for egg development was normal, as was larval survival, development time to stage IV, and morphology of stage IV larvae. However, in one instance in which eggs were immersed for 10 min in freshwater near the end of the development period (eye index 520 μm), about 1500 prelarvae died before molting to stage I; the other 14 000 larvae developed normally.

Effect of freshwater immersion on osmoregulation of adult lobster

The osmoregulatory ability of lobster was tested in a dilute medium of 500 mosm·kg^{-1} (17‰) salinity. Control lobsters were slight hyperregulators, maintaining their hemolymph osmotic pressure 130–140 mosm·kg^{-1} above that of the medium (Fig. 4). Lobster previously immersed in freshwater for 5 min did not exhibit a significant difference in their ability to hyperregulate. This ability was significantly lowered in lobster immersed for 10 and 15 min, even 7 d later and to a lesser extent 25 d after the immersion.

Discussion

Pseudocarcinonemertes homari displayed a wide tolerance to changing salinity. In general, although there was considerable individual variation, nemerteans survived for 4 d in media

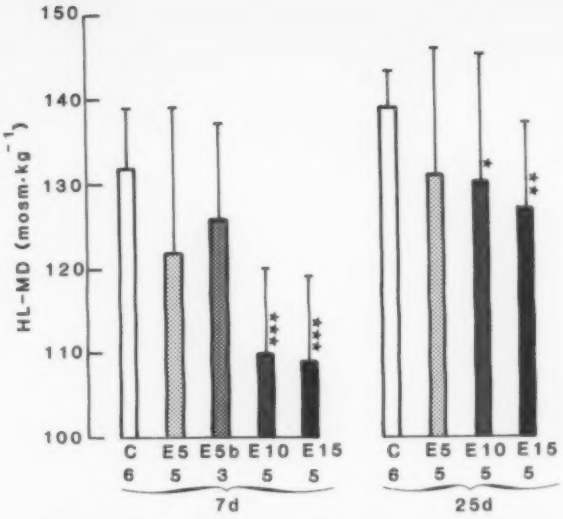


FIG. 4. Differences between the osmotic pressures of lobster hemolymph and medium (HL − MD) in a 500 mosm·kg^{-1} (17‰) medium at 9°C. C, controls; E5, E10, and E15, male and female lobsters previously immersed in freshwater for 5, 10, and 15 min, respectively; E5b, idem, using ovigerous females; 7 d and 25 d, number of days after immersion in freshwater. The number of animals is given in each group; 95% confidence intervals are indicated. Comparison with controls, *t*-test: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

ranging from 11 to 45‰ at 7°C; at 14.5°C the survival range was significantly reduced to between 19 and 37‰.

The salinity tolerance of *P. homari* was not due to a strong capacity to osmoregulate. It hyperosmoconformed and maintained a difference of about 30 mosm·kg^{-1} between the osmotic pressure of the ECF and the medium. Osmotic equilibration of the ECF in dilute media occurred in about 30 min, indicating that the nemertean integument was highly permeable to water. Tolerance to a wide range of salinities has been found in other nemerteans; *Lineus ruber* survived between 30 and 150% of seawater, i.e. between 10 and 51‰ at 10–15°C (Ling 1969), and in the Archinemertina *Procephalothrix spiralis*, mortality began to occur at 9.6‰ and all nemerteans died within 3 d at 5‰ at 7°C (Ferraris and Norenburg 1988).

Previous studies established that nemerteans are able to regulate their volume in dilute media (Lechenault 1965a, 1965b; Ferraris 1985b), and the few studies on hydromineral metabolism revealed nemerteans to be conformers. *Paranemertes peregrina* is a hypoosmotic conformer and its ECF is less concentrated than the external medium by about 50 mosm·kg^{-1} (Ferraris 1985a). *Lineus ruber* is able to slightly hyporegulate its ECF Na concentration (Ling 1971). The most studied species, *P. spiralis*, is a hyperosmotic conformer, maintaining its ECF osmotic pressure 20–60 mosm·kg^{-1} above that of dilute media and requiring about 15–30 min for osmotic adaptation to dilute media (Ferraris and Schmidt-Nielsen 1982; Ferraris 1984; Ferraris and Norenburg 1988). As demonstrated in *P. spiralis*, osmoregulation reflects the ionic regulation in ECF and is part of the volume regulation of the nemertean in dilute media by the elimination of Na and Cl (Ferraris and Schmidt-Nielsen 1982). Thus, our study on *P. homari* and results in other species show that nemerteans are tolerant of fluctuations in salinity and are weak osmoregulators that hyper- (and sometimes hypo-) osmoconform. The results support the hypothesis

that an efficient mechanism of intracellular isosmotic regulation exists in nemerteans.

That *P. homari* is tolerant of low salinities does not mean that it is widely euryhaline. In the wild, considerable differences in the prevalence and intensity of *P. homari* are found in lobster from different geographic areas. Although the reasons for these differences are unknown, it was speculated that salinity was a factor (Brattey et al. 1985). In *Carcinonemertes errans*, an egg predator of *Cancer magister*, large differences in infestation levels were found between crabs in the Pacific Ocean (79%) and those in the Columbia River estuary (6%) (McCabe et al. 1987). According to these authors, low salinities in the estuary were the major cause of the lower infestation, and Wickham (cited in McCabe et al. 1987) found that freshwater killed *C. errans* in 1–2 min.

Our results demonstrate that *P. homari* cannot survive in salinities below approximately 10‰. Immersion in freshwater was lethal in 4 min or less depending on temperature, even when the nemerteans were returned to seawater to recover.

Lobster embryos survived from 2 to 8 h when immersed in freshwater. The least tolerant stages were early eyed embryos and those about to hatch, but even these stages survived about 30 times longer in freshwater than did nemerteans. This is due to the relatively impermeable outer egg membrane that prevents rapid loss of ions from and invasion of water into the embryos (Charmantier and Aiken 1987, and unpubl. data). Immersion of lobster embryos in freshwater for up to 30 min generally did not interfere with embryonic development, hatching, larval development, or metamorphosis to the fourth stage.

Immersing the abdomen of ovigerous females in freshwater for up to 30 min was tolerated by the lobster. However, *P. homari* are sometimes found in large numbers in the branchial chamber of lobsters (Aiken et al. 1985), so the cephalothorax must also be immersed. Caution must be used in immersing lobster in freshwater, as the gills are the primary zones of ion and water exchange. However, a 5-min freshwater immersion does not cause mortality and did not affect the lobster's ability to osmoregulate in dilute media. Longer immersions (10–15 min) caused the death of some lobster and survivors had a decreased ability to hyperregulate that was only partially reversed after several weeks in normal salinities.

In summary, nemerteans were killed after 4 min in freshwater, lobster eggs survived at least 2 h in freshwater, and lobsters survived a 5-min immersion in freshwater and were able to tolerate at least a 30-min immersion of just the abdomen. Development of lobster eggs was not affected by a 30-min immersion in freshwater, and lobster osmoregulation was not affected by a 5-min immersion of the entire lobster. These results suggest that the nemertean *P. homari* could be eliminated from American lobster by immersing the abdomen of the lobster in freshwater for 5 min and then immersing the entire lobster for an additional 5 min. The temperature of the freshwater should be similar to that of seawater so that temperature shock is not added to the osmotic shock. Freshwater treatment should be avoided in lobster carrying eggs with eye indices >500 μm.

Preliminary experiments indicated that nemertean eggs would also be destroyed by this method. However, it is possible that some eggs might survive, hatch, and reinfest the lobster. Nemertean eggs develop in about 60 d, and the corresponding individuals reach sexual maturity within 40 d (Fleming and Gibson 1981). Thus, if nemerteans reappear after freshwater treatment, the treatment should be repeated within a month to eliminate them before they reproduce.

Acknowledgments

We thank W. Young-Lai, D. Graham, R. Chandler, and J. Parsons for their assistance and V. Zitko and R. Mounet-Guillaume for their help in data processing. Part of this study was supported by a grant from NATO.

References

AIKEN, D. E., S. L. WADDY, AND L. S. UHAZY. 1985. Aspects of the biology of *Pseudocarcinonemertes homari* and its association with the American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 42: 351–356.

AMERONGEN, H. M., AND F. S. CHIA. 1983. The role of nemertean cerebral organs in salinity stress tolerance re-examined in *Paranemertes peregrina* Coe (Hoplonemertea: Monostilifera). Mar. Behav. Physiol. 10: 1–22.

BRATTEY, J., A. CAMPBELL, A. E. BAGNALL, AND L. S. UHAZY. 1985. Geographic distribution and seasonal occurence of the nemertean *Pseudocarcinonemertes homari* on the American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 42: 360–367..

CAMPBELL, A., AND J. BRATTEY. 1986. Egg loss from the American lobster, *Homarus americanus*, in relation to nemertean, *Pseudocarcinonemertes homari*, infestation. Can. J. Fish. Aquat. Sci. 43: 772–780.

CHARMANTIER, G., AND D. E. AIKEN. 1987. Osmotic regulation in late embryos and prelarvae of the American lobster *Homarus americanus* H. Milne-Edwards, 1837 (Crustacea, Decapoda). J. Exp. Mar. Biol. Ecol. 109: 101–108.

CHARMANTIER, G., M. CHARMANTIER-DAURES, AND D. E. AIKEN. 1984. Neuroendocrine control of hydromineral regulation in the American lobster *Homarus americanus* H. Milne-Edwards 1837 (Crustacea, Decapoda). I. Juveniles. Gen. Comp. Endocrinol. 54: 8–19.

FERRARIS, J. D. 1984. Volume regulation in intertidal *Procephalothrix spiralis* (Nemertina) and *Clitellio arenarius* (Oligochaeta). II. Effects of decerebration under fluctuating salinity conditions. J. Comp. Physiol. 154B: 125–137.

1985a. Putative neuroendocrine devices in the Nemertina. An overview of structure and function. Am. Zool. 25: 73–85.

1985b. Effects of ablation of the cerebral organs and cerebral ganglia on volume regulation in an intertidal nemertine *Paranemertes peregrina*. Physiol. Zool. 58: 117–128.

FERRARIS, J. D., AND J. L. NORENBURG. 1988. Water and solute regulation in *Procephalothrix spiralis* Coe and *Clitellio arenarius* (Müller). Long-term acclimation to diluted seawaters and effect of putative neuroendocrine structures. Hydrobiologia 156: 161–173.

FERRARIS, J. D., AND B. SCHMIDT-NIELSEN. 1982. Volume regulation in an intertidal nemertine, *Procephalothrix spiralis* Coe. I. Short-term effects and independence on decerebration. J. Exp. Zool. 224: 307–319.

FLEMING, L. C., AND R. GIBSON. 1981. A new genus and species of monostiliferous hoplonemerteans, ectohabitant on lobsters. J. Exp. Mar. Biol. Ecol. 52: 79–93.

FRICK, J. H., AND J. R. SAUER. 1973. Examination of a biological cryostat/nanoliter osmometer for use in determining the freezing point of insect hemolymph. Ann. Entomol. Soc. Am. 66: 781–783.

HOLLELAND, T., AND H. J. FYHN. 1986. Osmotic properties of eggs of the herring *Clupea harengus*. Mar. Biol. 91: 377–383.

HUGHES, J. T., R. A. SHLESER, AND G. TCHOBANOGLOUS. 1974. A rearing tank for lobster larvae and other aquatic species. Prog. Fish-Cult. 36: 129–132.

KURIS, A. M., AND D. E. WICKHAM. 1987. Effect of nemertean egg predators on crustaceans. Bull. Mar. Sci. 41: 151–164.

LECHENAULT, H. 1965a. Neurosécrétion et osmorégulation chez les Lineidae (Hétéronémertes). C.R. Acad. Sci. Paris 261: 4868–4871.

1965b. Osmorégulation et neurosecrétion chez les Lineidae (Némertes). Gen. Comp. Endocrinol. 5: 695.

LIEBERMAN, H. R. 1983. Estimating LD50 using the probit technique: a basic computer program. Drug Chem. Toxicol. 6: 111–116.

LING, E. A. 1969. The structure and function of the cephalic organ of a nemertine *Lineus ruber*. Tissue Cell 1: 503–524.

1970. Further investigations on the structure and function of cephalic organ of a nemertine *Lineus ruber*. Tissue Cell 2: 569–588.

1971. The proboscis apparatus of the nemertine *Lineus ruber*. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 262: 1–22.

MCCABE, G. T. JR., R. L. EMMETT, T. C. COLEY, AND R. J. MCCONNELL. 1987. Effect of a river-dominated estuary on the prevalence of *Carcino-*

mertes errans, an egg predator of the Dungeness crab, *Cancer magister*. U.S. Dep. Commer. Fish. Bull. 85: 140–142.

PERKINS, C. P. 1972. Development rates at various temperatures of embryos of the northern lobster (*Homarus americanus* Milne-Edwards). U.S. Dep. Commer. Fish. Bull. 70: 95–99.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

WADDY, S. L., AND D. E. AIKEN. 1985. Immunofluorescent localization of American lobster egg yolk protein in the alimentary tract of the nemertean *Pseudocarcinonemertes homari*. Can. J. Fish. Aquat. Sci. 42: 357–359.

ZITKO, V. 1982. Letcur, the lethality curve program. Can. Tech. Rep. Fish. Aquat. Sci. 1134: 10 p.

Growth and Reproductive Energetics of three Scallop Species from British Columbia (*Chlamys hastata, Chlamys rubida*, and *Crassadoma gigantea*)

B. A. MacDonald and R. J. Thompson

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Nfld. A1C 5S7, Canada

and N. F. Bourne

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

MacDonald, B. A., R. J. Thompson, and N. F. Bourne. 1991. Growth and reproductive energetics of three scallop species from British Columbia (*Chlamys hastata*, *Chlamys rubida*, and *Crassadoma gigantea*). Can. J. Fish. Aquat. Sci. 48: 215–221.

Three scallop species from British Columbia display different strategies for partitioning available energy between somatic tissue growth and gamete production as they increase in age. The spiny scallop *Chlamys hastata* and the pink scallop *Chlamys rubida* only live about 6 yr and rarely exceed 80 mm in shell height whereas the rock scallop *Crassadoma gigantea* may reach 170 mm in height and live for 20 yr or more. Growth, reproductive output, and reproductive effort at any given age are higher in *Chlamys hastata* than in the smaller *Chlamys rubida*. Somatic growth in *Crassadoma gigantea* ceases completely in the final years, but in the short-lived species *Chlamys hastata* and *Chlamys rubida*, individuals continue to grow until they die. In long-lived pectinids the emphasis often shifts from somatic growth to gamete production before the midpoint of the life cycle, and our observations on *Crassadoma gigantea* are consistent with this trend. Short-lived species, however, invest relatively less in reproduction; in our study, reproductive output in *Chlamys rubida* did not exceed 40% of nonrespired assimilation (net production), and reproductive effort in *Chlamys hastata* did not reach 50% until the final year of life.

Selon l'âge, trois espèces de pétoncle de la Colombie-Britannique utilisent différentes stratégies de répartition de l'énergie disponible entre la croissance des tissus somatiques et la production de gamètes. La durée de vie du pétoncle épineux *Chlamys hastata* et du pétoncle rose *Chlamys rubida* se situe à environ 6 ans et la hauteur de leur coquille dépasse rarement 80 mm; par contre, le pétoncle des roches *Crassadoma gigantea* peut vivre 20 ans ou plus et sa coquille peut atteindre 170 mm de hauteur. Le taux de croissance, l'efficacité de la reproduction et le niveau d'énergie consacrée à la reproduction à un âge donné sont plus élevés chez *Chlamys hastata*, que chez *Chalmys rubida*, de plus petite taille. Au cours des dernières années du cycle vital, la croissance des tissus somatiques cesse complètement chez *Crassadoma gigantea*, mais chez *Chlamys hastata* et *Chlamys rubida*, espèces dont la durée de vie est courte, la croissance se poursuit jusqu'à la mort. Chez les pectinidés à longue durée de vie, la production de gamètes prend souvent plus d'importance que la croissance des tissus somatiques avant le milieu du cycle vital; dans le cas de *Crassadoma gigantea*, nos observations confirment cette tendance. Toutefois, les espèces à courte durée de vie consacrent relativement moins d'énergie à la reproduction; au cours de notre étude, l'efficacité de la reproduction chez *Chlamys rubida* n'a pas dépassé 40 pour cent de l'assimilation en l'absence de respiration (production nette) et chez *Chlamys hastata*, le niveau d'énergie consacrée à la reproduction n'a atteint 50% qu'au cours de la dernière année du cycle vital.

Received January 10, 1990
Accepted August 22, 1990
(JA432)

Reçu le 10 janvier 1990
Accepté le 22 août 1990

The pink scallop *Chlamys rubida* (Hinds, 1845), spiny scallop *Chlamys hastata* (Sowerby, 1843), and rock scallop *Crassadoma gigantea* (Gray, 1825; formerly *Hinnites* Bernard, 1986) all occur exclusively on the Pacific coast of North America from southern California to Alaska (Abbott 1974; Bernard 1983a). *Crassadoma gigantea* is usually found attached to hard substrates from the lower part of the intertidal zone to water depths of 60 m whereas *Chlamys hastata* occurs subtidally (5–150 m) in areas of strong current on hard bottoms (gravel or rock) and *Chlamys rubida* is confined to softer bottoms (5–200 m) (Bourne 1987). Sexes are separate and there are no external signs of sexual dimorphism in any of the three species.

Local scallop populations do not occur in large or dense beds, and commercially exploited scallop resources in British Columbia are therefore restricted to a few minor fisheries, such as those which have been established for *Chlamys rubida* and *Chlamys hastata* (especially the latter) in southern British Columbia since 1982 (Bourne 1987). Patchy spatial distribution and harvesting difficulties have prevented the development of a commercial fishery for *Crassadoma gigantea* in British Columbia, although a recreational fishery does exist.

There are published studies on the reproductive cycle of *Crassadoma gigantea* (Lauren 1982; Malachowski 1988) and its growth rate (Leighton 1979; Monical 1980; MacDonald and Bourne 1989), usually in relation to its potential value for aqua-

culture (Leighton and Phleger 1977). The basic biological information essential for fisheries management is lacking for the *Chlamys* spp., however, and the paucity of data on population size and structure, growth rate, mortality, distribution, and spawning results in the two species being managed as one (Bourne and Harbo 1987). In this paper we describe growth rates and age-related changes in the partitioning of nonrespired assimilated energy between shell growth, somatic production, and reproductive output for natural populations of *Chlamys rubida*, *Chlamys hastata*, and *Crassadoma gigantea* from British Columbia. Seasonal gametogenic activity and its relationship with biochemical energy reserves in these scallops will be considered elsewhere.

Materials and Methods

Sample Collections

Samples (10–80 individuals) of *Chlamys hastata* and *Crassadoma gigantea* were collected monthly from natural populations near Bamfield, B.C., while *Chlamys rubida* were obtained near Denman Island. Complete size ranges of scallops were obtained from water depths of 5–25 m by SCUBA divers throughout 1985 and 1986. *Crassadoma gigantea* had a more restricted local distribution which occasionally resulted in a reduction of sample size and sampling frequency.

The gonad was separated from the remaining soft body tissues (somatic) and the shell. For each individual scallop, these three components were weighed separately after drying at 80°C for 48 h. The gonads of *Chlamys hastata* and *Crassadoma gigantea* consisted not only of the typical tongue-shaped structure characteristic of pectinids, but also of follicles surrounding the digestive gland. In order to calculate total gonad weight for these species it was first necessary to establish the relationship between digestive gland weight and shell height by carefully dissecting the digestive gland from the surrounding gonadal mass for a complete size range in each species. For each individual scallop in subsequent samples, the weight of the digestive gland predicted from the regression equation (digestive gland weight against shell height) was then subtracted from the observed weight of the digestive gland/gonad assemblage and added to the somatic weight, the difference representing the weight of the gonad.

Growth Rates

In many bivalves the ages of individuals have often been determined by counting annual growth rings on the exterior surface of the shell in order to construct a growth curve for the population. This technique has proved successful for several species of pectinids, including *Placopecten magellanicus* (Stevenson and Dickie 1954), *Pecten maximus* (Mason 1957), *Patinopecten caurinus* (Silina 1978), and *Swiftopecten swifti* (Ponurovski and Silina 1983). Individual age has also been determined in *Placopecten magellanicus* (Merrill et al. 1966: MacDonald and Thompson 1985a) and *Chlamys islandica* (Johannessen 1973) using the annual growth increments on the calcareous portion of the ligament. In this study, age estimates for individual *Chlamys hastata* and *Chlamys rubida* were obtained from the ligament and from external growth lines formed annually in January or February (Bourne and Harbo 1987; A. V. Silina, Academy of Sciences, Vladivostock, USSR, pers. comm.). The irregular surface of the shell of *Crassadoma gigantea* prevents age determination using traditional growth ring analysis, but counting the lines on the ligament is a reliable alternative (MacDonald and Bourne 1989).

Shell height, the maximum distance between the dorsal (hinge) and ventral margins (Seed 1980), was recorded to the nearest 0.1 mm using vernier calipers. The relationship between shell height and age was described using the von Bertalanffy model

$$H_t = H_\infty[1 - e^{-K(t - t_0)}]$$

where H_t is shell height at time t, H_∞ is the mean asymptotic shell height, K is the Brody growth coefficient, and t_0 is a parameter representing time when shell height equals zero (Ricker 1975). Von Bertalanffy curves were fitted by the Marquardt algorithm within the NLIN procedure of the Statistical Analysis System (SAS Institute Inc.). The data for *Crassadoma gigantea* have been published elsewhere (MacDonald and Bourne 1989).

For *Chlamys hastata* and *Chlamys rubida* the mean somatic weight was calculated directly for each age-class. Owing to the greater number of age-classes in *Crassadoma gigantea*, a few having poor representation (less than three individuals), the mean somatic weight for each age-class in this species was estimated after fitting the data to the polynomial equation

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 \ldots \beta_m x^m + \epsilon$$

where $\beta_0, \beta_1, \ldots \beta_m$ are population parameters, y is the predicted weight for a given age, and ϵ is random error associated with x.

The allometric equation $y = ax^b$ (where y is the predicted weight at shell height x and a and b are fitted parameters) was used to describe the relationships between gonad weight or shell weight and shell height in each species. Both variates were transformed to logarithms ($\log_{10}$) before fitting the data to a straight line by least squares regression in the General Linear Model (GLM) of the Statistical Analysis System (SAS Institute Inc.). Comparison of regressions was carried out by analysis of covariance (ANCOVA), using the GLM in SAS. For cases in which the slopes were homogeneous, we tested for differences between elevations. For each age-class, shell height was obtained from the von Bertalanffy equation, and the corresponding gonad weight was derived from regressions of gonad weight against shell height.

Production

Several major components contribute to total annual production in scallops. These include energy released as gametes (P_r), energy committed to somatic tissue growth (P_g), and energy incorporated into the organic matrix of the shell (P_s). The incremental change in somatic tissue weight or shell weight W between consecutive year-classes ($W_{(x+1)} - W_x$) was used to estimate somatic production (P_g) and shell production (P_s), respectively. Proximate biochemical analysis revealed little interspecific difference in the total energy content of somatic tissue, so a mean value of 1 g = 20.0 kJ was used for all three species (unpublished data). The organic content of the shell was determined by weight loss on ignition for 18 h at 450°C (shells from 10 animals for each species over the entire size range), and annual deposition of organic matter was calculated from growth curves. P_s was then estimated by converting these values to energy units (1 g conchiolin = 21.2 kJ; Hughes 1970).

Gamete production (P_r) for each age-class was estimated as the weight loss of the gonad upon spawning, determined from

logarithmic regressions of gonad weight on shell height in the months before and after spawning. Unlike the weathervane scallop *Patinopecten caurinus*, which spawns in May in this area (MacDonald and Bourne 1987), these three pectinids display asynchronous seasonal reproductive activity and may not completely empty the gonad during spawning, which makes it difficult to estimate reproductive output. *Chlamys hastata* and *Crassadoma gigantea* both appear to spawn in July or August whereas *Chlamys rubida* spawns twice a year, first in March or April and then in September or October, making it necessary to estimate total P_r as the sum of the outputs from the two spawning periods ($P_r = P_{r_1} + P_{r_2}$). Asynchrony is particularly problematical in *Crassadoma gigantea*, so for this species we calculated a gonadosomatic index (dry weight of gonad/dry weight of somatic tissue) for each scallop in the data set. All individuals which were obviously spent had an index less than 0.2 whereas those which appeared not to have spawned at all had an index greater than 0.3. Reproductive output was therefore determined for *Crassadoma gigantea* of given ages by subtracting the dry weights of the gonads in the former group from those in the latter, making use of the logarithmic regressions of gonad weight against shell height. For all species the weight of gametes released was converted to energy equivalents using a factor of 1 g = 20.8 kJ, calculated from proximate biochemical analysis for all three species (unpublished data). Comparison of regressions by ANCOVA showed that in each species the weights of somatic tissue, shell, and gonad did not differ consistently between male and female scallops at the 5% significance level, and data for both sexes were therefore combined to estimate the various components of production.

The proportion of total net production allocated to gametes is referred to as reproductive effort (RE) and was calculated as $RE = P_r \times 100/P_t$ where P_t is total production ($P_r + P_g + P_s$). The turnover ratio, or production to biomass ratio, was calculated for each species as (P_t/B) where B is the energy equivalent (kilojoules) of the mean somatic weight or biomass for an individual in a given age-class. Values for P_r, P_g, and P_s were obtained from the appropriate regression equations, i.e. they represent predicted rather than mean values. To compare statistically RE and turnover ratio between species, mean values for RE and P_t/B were calculated for each age-class based on mean values of the underlying variables P_g, P_r, P_s, and B rather than predicted values. For the age-classes in which data were available for all three species (i.e. 2–6 yr), nonparametric linear regressions were calculated between RE and age and between turnover ratio and age, using ranked means for the dependent variables (Iman and Conover 1979). Comparisons of regressions were then carried out using ANCOVA, as described previously.

Results

Growth

Individual rock scallops, *Crassadoma gigantea*, may live for over 20 yr and grow to 175 mm in shell height (MacDonald and Bourne 1989), but *Chlamys hastata* and *Chlamys rubida* only live for 6 yr and rarely exceed 80 and 70 mm in shell height, respectively (Fig. 1A, 2A). After the age of 2 yr, mean shell height and somatic weight are greater in *Chlamys hastata* than in *Chlamys rubida* (Fig. 1A, 1B). We recorded higher values for the Brody growth coefficient K in the two species of *Chlamys* than in *Crassadoma gigantea*, indicating that the

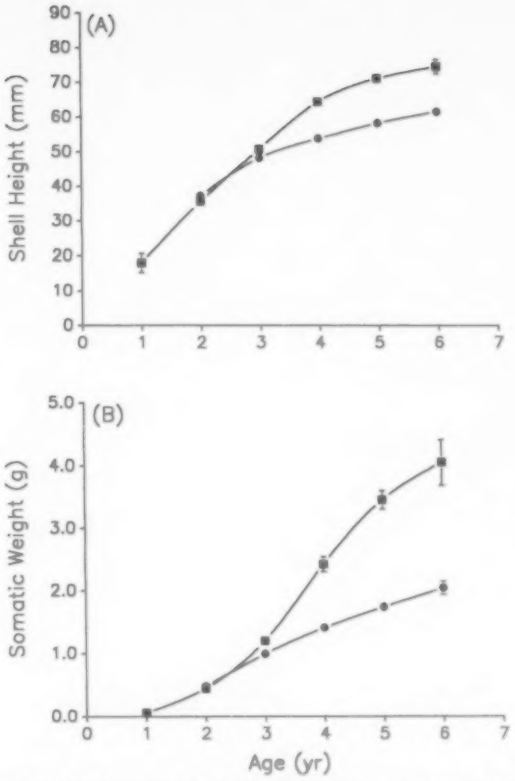


FIG. 1. (A) Shell height and (B) dry weight of somatic tissue for each age-class in *Chlamys hastata* (■) and *Chlamys rubida* (●). Means ± 95% confidence intervals. For points lacking a vertical bar, the 95% confidence intervals are smaller than the symbols. Parameters for the von Bertalanffy equations describing shell growth are given in Table 1.

Chlamys spp. reach their predicted mean asymptotic heights much more rapidly than does the larger *Crassadoma gigantea* (Table 1). The von Bertalanffy model fitted the data for *Chlamys hastata* less well than it did for the other species, and the predicted mean asymptotic height of 93.9 mm for *Chlamys hastata* may be an overestimate resulting from low sample numbers in the oldest age-class because the largest specimen collected was 80.5 mm in height. Predicted asymptotic heights for *Chlamys rubida* and *Crassadoma gigantea*, however, were very similar to the maximum values recorded.

Production of Shell and Somatic Tissue

In all three species, annual somatic production (P_g) increased consistently for the first few age-classes before reaching a maximum and then declining (Fig. 2B, 3). Negative values were obtained for very old individuals of *Crassadoma gigantea* (Fig. 2B), indicating that these senescent rock scallops may have resorbed somatic tissue, as reported for *Placopecten magellanicus* (MacDonald and Thompson 1985a). The value for P_g was greater in *Chlamys hastata* than in *Chlamys rubida*, with a maximum reached at age 4 or 5 yr in the former species and at 2 yr in the latter (Fig. 3). The production of shell material (P_s) showed the same trends as P_g in all three species, although values were lower than for P_g, and P_s was never negative. Greater P_s values were reported for *Crassadoma gigantea* than for individuals of equivalent size or age from the other two

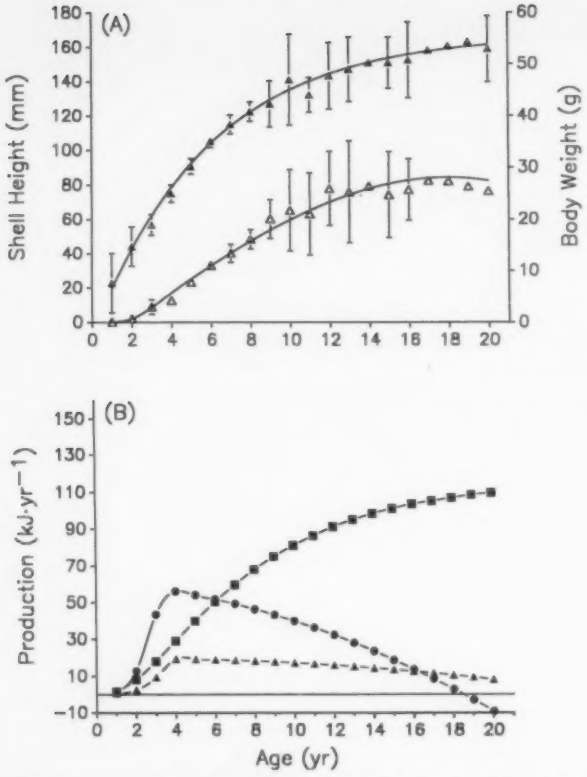


FIG. 2. (A) Age-specific shell heights (▲) (from MacDonald and Bourne 1989) and somatic weights (△) for *Crassadoma gigantea*. Mean values (± 95% confidence intervals for age-classes containing more than three individuals). Parameters for the von Bertalanffy equation describing shell growth are listed in Table 1. Growth of somatic tissue is given by a polynomial equation (see Materials and Methods) $\beta_0 = 4.29$; $\beta_1 = 2.81$; $\beta_2 = -0.05$; $\beta_3 = -0.002$; $n = 121$; $r^2 = 0.95$; $P < 0.001$, where y = somatic weight (g) and x = age (yr). (B) Annual production of somatic tissue (P_g, ●), shell (P_s, ▲), and gametes (P_r, ■) in *Crassadoma gigantea*.

smaller species because *Crassadoma gigantea* possesses a much heavier and thicker shell. For example, the shell of a *Crassadoma gigantea* 62 mm in height weighed 30.5 g whereas shells of a 63-mm *Chlamys hastata* and a 61-mm *Chlamys rubida* weighed only 11.2 and 10.6 g, respectively.

Gonad Production and Reproductive Effort

In all cases, gonad dry weight was positively correlated with shell height (Table 2). Reproductive output (P_r), the difference between the energy content of the prespawning and postspawning gonad, increased with age for all three species (Fig. 2B, 3). In *Crassadoma gigantea* and *Chlamys rubida*, but not in *Chlamys hastata*, the P_r curve approached an asymptote. Although each individual *Chlamys rubida* spawned twice, its cumulative gamete production was far less than that of a *Chlamys hastata* of the same age or size, so that a 4-yr-old *Chlamys hastata* 63 mm in height had a P_r of 10.2 kJ whereas a 6-yr-old *Chlamys rubida* 61 mm in height had an output of only 4.0 kJ (Fig. 3).

Reproductive effort (RE) was an increasing function of age for all species, and the emphasis shifted from growth of soma and shell in the earliest years to the production of gametes in later years (Fig. 4A). Gamete production did not exceed somatic growth in *Chlamys hastata* until the last year of life (i.e. RE was less than 50%), and in *Chlamys rubida*, RE reached a maximum of only 37%. In *Crassadoma gigantea*, however, values for RE exceeded 90% in the oldest individuals, and the shift from growth to reproduction occurred at 8 yr of age. Over the age range (2–6 yr) for which data were available for all three species, there was no significant difference in RE between species (no differences between either slopes or elevations in nonparametric linear regressions of ranks of mean observations; $P > 0.15$ in each case).

Turnover Ratio

In each species, turnover ratio (P_r/B) declined steadily with age (Fig. 4B), although in *Crassadoma gigantea* the decrease was slow in older individuals. Over the age range for which data were available for all three species, the slopes of the nonparametric linear regressions on ranked means were not significantly different ($P = 0.49$), so a common slope was fitted. Further analysis (ANCOVA) demonstrated that the elevations were significantly different ($P = 0.018$), i.e. turnover rates varied between species. Despite their larger size, individuals of *Crassadoma gigantea* were more productive per unit biomass than those of either of the *Chlamys* species.

Discussion

Within the Pectinidae there is a great deal of diversity in growth rates and longevity, some species living as little as 14–18 mo (e.g. *Argopecten irradians*), others for over 20 yr (e.g. *Placopecten magellanicus*, *Chlamys islandica*, *Patinopecten caurinus*). The pink scallop *Chlamys rubida* from British Columbia has approximately the same shell growth rate as *Chlamys opercularis* from the Clyde Sea area (Taylor and Venn 1978) and grows slightly faster than *Chlamys varia* from the Bay of Brest (Conan and Shafee 1978). The spiny scallop *Chlamys hastata* is relatively fast growing in British Columbia, reaching a shell height of 80 mm in 6 yr, whereas *Chlamys islandica* from Norway takes 15 yr to attain this size and may live for 23 yr (Vahl 1981a), which appears to be exceptional for this genus. Pink and spiny scallops from British Columbia grow at rates comparable with those reported for other species of *Chlamys*, excepting *Chlamys islandica*. Neither *Chlamys*

TABLE 1. Summary of parameters and 95% confidence intervals for the relationship between shell height (mm) and age (yr) fitted to the von Bertalanffy model $H_t = H_\infty [1 - e^{-K(t-t_0)}]$ (see text for details) in *Chlamys hastata*, *Chlamys rubida*, and *Crassadoma gigantea*.

	H_∞	K	t_0	n	r^2
Chlamys hastata	93.7 ± 5.2	0.32 ± 0.05	0.46 ± 0.11	301	0.88
Chlamys rubida	67.0 ± 2.4	0.41 ± 0.07	−0.19 ± 0.31	697	0.97
Crassadoma gigantea	167.0 ± 4.2	0.17 ± 0.01	0.27 ± 0.23	121	0.96

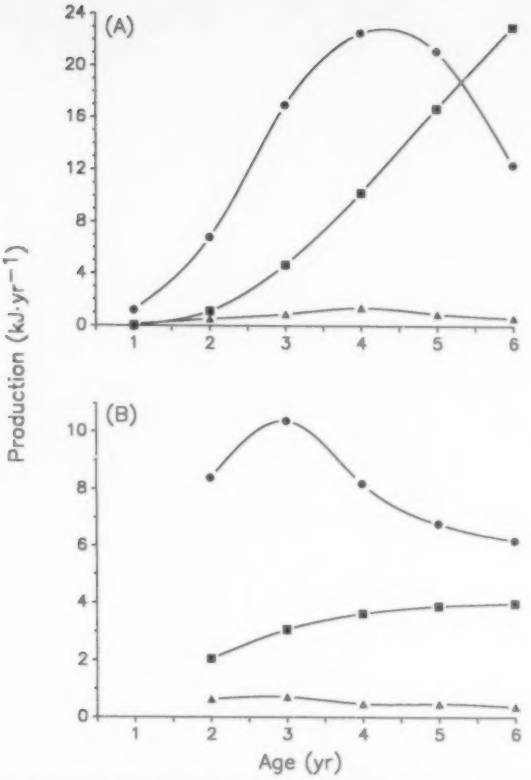


FIG. 3. Annual production of somatic tissue (P_s, ●), shell (P_s, ▲), and gametes (P_r, ■) for each age-class of (A) *Chlamys hastata* and (B) *Chlamys rubida*.

rubida nor *Chlamys hastata* commonly exceeds 80 mm in shell height, and both live for less than 7 yr, making them typical representatives of the genus *Chlamys*.

The rock scallop *Crassadoma gigantea* is unusual in that it has abandoned the free-swimming habit normally associated with pectinids and adheres permanently by cementing the shell to rocky substrates when it reaches approximately 20–30 mm in height (Yonge 1951). When grown suspended in the water column under culture conditions, *Crassadoma gigantea* from California attains a shell height of 120 mm in 2 or 3 yr (Leighton and Phleger 1977). In British Columbia it requires approximately 8 yr of growth under natural conditions to reach this size. *Crassadoma gigantea* grows at a slower rate than another large species, *Patinopecten caurinus* (weathervane scallop), from the same area, although it attains a slightly larger size (MacDonald and Bourne 1987), but *Crassadoma gigantea* has a growth rate similar to that of the giant scallop *Placopecten magellanicus* from some locations on the east coast of Canada (MacDonald and Thompson 1985a). Slow growth and a large investment of energy in shell production may limit the mariculture potential of *Crassadoma gigantea* in British Columbia (Bernard 1983b).

The partitioning of available energy between growth and reproduction as the animal increases in age has a significant effect on survivorship, rate of maturation, fecundity, growth rate, and longevity. Reproductive output (P_r) increases with age in *Chlamys rubida*, but unlike P_r in most other iteroparous pectinid species, including *Chlamys hastata* (this study) and *Chlamys varia* (Shafee and Lucas 1982), it never exceeds the production of somatic tissue. Gamete production is greater than somatic production in *Chlamys hastata* in the final year of life whereas the transition from growth to reproduction occurs between the ages of 3 and 5 yr in *Chlamys varia*, depending on annual variation in P_r. Reproductive output for *Chlamys rubida* is within the range of P_r values described for *Chlamys varia* studied in the northwest of France for two consecutive years (Shafee and Lucas 1982). Peak somatic production occurs at age 2 yr for both *Chlamys rubida* and *Chlamys varia*, with maximum P_s being approximately twice as high in the former than in the latter. Shell production, however, is greater in *Chlamys varia* than in *Chlamys rubida*.

Trends in age-specific production of soma, gonad, and shell in *Crassadoma gigantea* are similar to those described for other long-lived pectinids such as *Chlamys islandica* (Vahl 1981a), *Placopecten magellanicus* (MacDonald and Thompson 1985b), and *Patinopecten caurinus* (MacDonald and Bourne 1987). Maximum P_s is observed between the ages of 4 and 5 yr for all these species and for *Chlamys hastata* from British Columbia. *Crassadoma gigantea* produces a much thicker and heavier shell than other species, resulting in greater values for P_s, although there is some evidence that this is not achieved at the expense of somatic production (MacDonald and Bourne 1989). The reproductive output of *Crassadoma gigantea* is similar to that of *Placopecten magellanicus* from a water depth of 20 m in Dildo and Colinet, Newfoundland, but less than in *Placopecten magellanicus* from 20 m at Sunnyside, Newfoundland. In contrast, P_r for *Crassadoma gigantea* is slightly greater than for *Patinopecten caurinus* from inshore in British Columbia. Our values for gamete production in *Chlamys hastata* are greater than any which have been recorded for the genus *Chlamys*, including species such as *Chlamys islandica* which may live almost four times as long as *Chlamys hastata*.

TABLE 2. Summary of regression parameters for the relationship between gonad dry weight y (g) and shell height x (mm) given by $\log y = \log a + b \log x$ for *Chlamys hastata*, *Chlamys rubida*, and *Crassadoma gigantea*. For *Crassadoma gigantea*, the prespawning sample was made up of all those individuals with a gonadosomatic index < 0.2 and the postspawning sample of all those individuals with an index > 0.3 (see Materials and Methods). All regressions were highly significant ($P < 0.001$).

	Log a	b	n	r^2	F
Chlamys hastata					
Gonad weight (prespawning) (June–July)	−7.56	4.14	60	0.92	713
Gonad weight (postspawning) (September–November)	−8.70	4.50	60	0.89	450
Chlamys rubida					
Gonad weight (prespawning) (January–February)	−5.96	3.08	153	0.25	51.4
(August–September)	−4.95	2.56	115	0.17	24.1
Gonad weight (postspawning) (April–May)	−7.26	3.78	72	0.29	28.8
(October–November)	−6.57	3.36	124	0.15	20.8
Crassadoma gigantea					
Gonad weight (prespawning)	−4.02	2.29	54	0.90	496
Gonad weight (postspawning)	−5.79	2.95	29	0.76	84.4

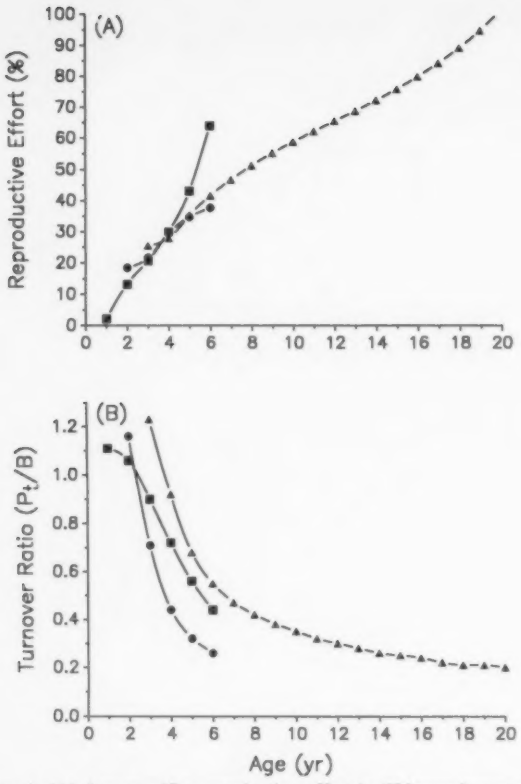


FIG. 4. (A) Age-specific reproductive effort in *Chlamys hastata* (■), *Chlamys rubida* (●), and *Crassadoma gigantea* (▲). (B) Mean turnover ratios ($(P_g + P_r + P_s/B$ where B = somatic biomass) for each age-class in the three species.

In all three species considered here, the steady decrease in turnover ratio with increasing age indicates that older scallops, despite their higher reproductive output, are less productive per unit body weight than younger conspecifics because an increasing proportion of the assimilated ration is required for maintenance as the scallop ages, leaving less energy available for production (MacDonald and Thompson 1986). Despite its larger size, *Crassadoma gigantea* is more productive per unit weight than the two *Chlamys* species from British Columbia. The turnover ratio may become independent of age in the final years for long-lived species such as *Placopecten magellanicus* (MacDonald and Thompson 1985b) and *Chlamys islandica* (Vahl 1981a) if production, principally P_r, increases as rapidly as body mass accumulates. Weight-specific production is greater in *Chlamys rubida* than in *Chlamys varia* (Shafee and Lucas 1982), largely as a result of greater somatic production by the former. *Chlamys hastata* is more productive than *Chlamys rubida* in any given age-class and has a higher turnover ratio, despite its larger body size.

This gradual shift of available energy to reproductive processes results in a reduction in growth and an increase in RE as the individual grows older. In the oldest individuals of *Crassadoma gigantea*, almost all available energy is spent on gametes, as in several species of bivalves, including long-lived pectinids (Vahl 1981b; Bayne and Newell 1983; Thompson 1984; MacDonald et al. 1987). Owing to low gamete production, which is often associated with continuing somatic growth during the final years of life, high values (>80%) for RE have not been found in short-lived scallop species such as *Chlamys varia* (Shafee and Lucas 1982), *Chlamys hastata*, and *Chlamys rubida* (this study). *Chlamys hastata* has a substantial gamete output for its size, and the sustained somatic production throughout life in this species is probably the reason that very high values of RE are not attained. In *Chlamys rubida*, maximum RE is less than 40%, owing to a combination of low P_r (despite two spawning periods per year) and relatively high P_g.

In conclusion, these three pectinid species from the same environment display different life histories and a variety of strategies for determining the allocation of available resources between growth and reproduction. According to published accounts and data from this study, short-lived iteroparous pectinids may continue to grow actively throughout life whereas in long-lived species growth ceases in senescent individuals and body tissue may be utilized to support gamete production.

Acknowledgements

We thank Dr. A. V. Silina from the Academy of Sciences of the USSR, Vladivostock, for confirming the formation and location of annual growth rings in pink and spiny scallops. Scallops were obtained from Canadian Benthic, Bamfield, British Columbia. C. Hodgson, W. Carolsfeld, and E. Downey assisted with laboratory measurements. M. Miller undertook some data analysis and prepared all the figures. Statistical advice was provided by C. Bajdik. This project was supported by a Canadian Government Laboratory Visiting Postdoctoral Fellowship to B.A.M. and NSERC operating grants to B.A.M. and R.J.T.

References

ABBOTT, R. T. 1974. American seashells: the marine Mollusca of the Atlantic and Pacific coasts of North America. Van Nostrand Reinhold Co., New York, NY. 663 p.

BAYNE, B. L., AND R. C. NEWELL. 1983. Physiological energetics of marine molluscs, p. 407–515. *In* A. S. M. Saleuddin and K. M. Wilbur [ed.] The Mollusca. Vol. 4(1). Academic Press Inc., New York, NY.

BERNARD, F. R. 1983a. Catalogue of the living Bivalvia of the eastern Pacific Ocean: Bering Strait to Cape Horn. Can. Spec. Publ. Fish. Aquat. Sci. 61: 102 p.

1983b. Physiology and the mariculture of some northeastern Pacific bivalve molluscs. Can. Spec. Publ. Fish. Aquat. Sci. 63: 1–24.

1986. *Crassadoma* gen. nov. for "*Hinnites*" *giganteus* (Gray, 1825) from northeastern Pacific Ocean (Bivalvia: Pectinidae). Venus Jpn. J. Malacol. 45: 70–74.

BOURNE, N. F. 1987. Scallops, p. 107–112. *In* R. M. Harbo and G. S. Jamieson [ed.] Status of invertebrate fisheries off the Pacific coast of Canada (1985/86). Can. Tech. Rep. Fish. Aquat. Sci. 1576: 158 p.

BOURNE, N. F., AND R. M. HARBO. 1987. Size limits for pink and spiny scallops, p. 113–122. *In* R. M. Harbo and G. S. Jamieson [ed.] Status of invertebrate fisheries off the Pacific coast of Canada (1985/86). Can. Tech. Rep. Fish. Aquat. Sci. 1576: 158 p.

CONAN, G., AND M. S. SHAFEE. 1978. Growth and biannual recruitment of the black scallop *Chlamys varia* (L.) in Lanveoc area, Bay of Brest. J. Exp. Mar. Biol. Ecol. 35: 59–71.

HUGHES, R. N. 1970. An energy budget for a tidal-flat population of the bivalve *Scrobicularia plana* (da Costa). J. Anim. Ecol. 39: 357–379.

IMAN, R. L., AND W. J. CONOVER. 1979. The use of the rank transform in regression. Technometrics 21: 499–509.

JOHANNESSEN, O. H. 1973. Age determination in *Chlamys islandica* (O. F. Mueller). Astarte 6: 15–20.

LAUREN, D. J. 1982. Oogenesis and protandry in the purple-hinge rock scallop, *Hinnites giganteus*, in upper Puget Sound, Washington, U.S.A. Can. J. Zool. 60: 2333–2336.

LEIGHTON, D. L. 1979. A growth profile for the rock scallop *Hinnites multirugosus* held at several depths off La Jolla, California. Mar. Biol. 51: 229–232.

LEIGHTON, D. L., AND C. F. PHLEGER. 1977. The purple-hinge rock scallop, a new candidate for marine aquaculture. Proc. World Maricult. Soc. 8: 457–469.

MACDONALD, B. A., AND N. F. BOURNE. 1987. Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia. Can. J. Fish. Aquat. Sci. 44: 152–160.

1989. Growth of the purple-hinge rock scallop, *Crassadoma gigantea* Gray, 1825 under natural conditions and those associated with suspended culture. J. Shellfish Res. 8: 179–186.

MACDONALD, B. A., AND R. J. THOMPSON. 1985a. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. I. Growth rates of shell and somatic tissue. Mar. Ecol. Prog. Ser. 25: 279–294.

1985b. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. II. Reproductive output and total production. Mar. Ecol. Prog. Ser. 25: 295–303.

1986. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. III. Physiological ecology, the gametogenic cycle and scope for growth. Mar. Biol. 93: 37–48.

MACDONALD, B. A., R. J. THOMPSON, AND B. L. BAYNE. 1987. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. IV. Reproductive effort, value and cost. Oecologia (Berlin) 72: 550–556.

MALACHOWSKI, M. 1988. The reproductive cycle of the rock scallop *Hinnites giganteus* (Gray) in Humboldt Bay, California. J. Shellfish Res. 7(3): 341–348.

MASON, J. 1957. The age and growth of the scallop, *Pecten maximus* (L.), in Manx waters. J. Mar. Biol. Assoc. U.K. 36: 473–492.

MERRILL, A. S., J. A. POSGAY, AND F. E. NICHY. 1966. Annual marks on shell and ligament of sea scallop (*Placopecten magellanicus*). Fish. Bull. 65(2): 299–311.

MONICAL, J. B. JR. 1980. Comparative studies on growth of the purple-hinge rock scallop *Hinnites multirugosus* (Gale) in the marine waters of Southern California. Proc. Natl. Shellfish. Assoc. 70: 14–21.

PONUROVSKI, S. K., AND A. V. SILINA. 1983. The determination of the age and linear growth rate of the scallop *Swiftopecten swiftii*. Biol. Morya 1: 20–24.

RICKER, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

SEED, R. 1980. Shell Growth and Form in the Bivalvia, p. 23–67. *In* D. C. Rhoads and R. A. Lutz [ed.] Skeletal growth of aquatic organisms: biological records of environmental change. Plenum Press, New York, NY.

SHAFEE, M. S., AND A. LUCAS. 1982. Variations saisonnières du bilan énergétique chez les individus d'une population de *Chlamys varia* (L.):Bivalvia, Pectinidae. Oceanol. Acta 5: 331–338.

SILINA, A. V. 1978. Determination of age and growth rate of the yezo scallop by the sculpture of its shell surface. Biol. Morya 5: 29–39.

STEVENSON, J. A., AND L. M. DICKIE. 1954. Annual growth rings and rate of growth of the giant scallop, *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. J. Fish. Res. Board Can. 11: 660–671.

TAYLOR, A. C., AND T. J. VENN. 1978. Growth of the queen scallop, *Chlamys opercularis*, from the Clyde Sea area. J. Mar. Biol. Assoc. U.K. 58: 687–700.

THOMPSON, R. J. 1984. Production, reproductive effort, reproductive value and reproductive cost in a population of the blue mussel *Mytilus edulis* from a subarctic environment. Mar. Ecol. Prog. Ser. 16: 249–257.

VAHL, O. 1981a. Energy transformations by the Iceland scallop, *Chlamys islandica* (O. F. Muller), from 70° N. I. The age-specific energy budget and net growth efficiency. J. Exp. Mar. Biol. Ecol. 53: 281–296.

1981b. Age-specific residual reproductive value and reproductive effort in the Iceland scallop, *Chlamys islandica* (O. F. Muller). Oecologia (Berlin) 51: 53–56.

YONGE, C. M. 1951. Observations on *Hinnites multirugosus* (Gale). Univ. Calif. Publ. Zool. 55: 409–420.

Downstream Effects of Mine Effluent on an Intermontane Riparian System

Johnnie N. Moore

Department of Geology, University of Montana, Missoula, MT 59812, USA

Samuel N. Luoma

U.S. Geological Survey, Water Resource Division, 345 Middlefield Road, Menlo Park, CA 94025, USA

and Donald Peters

Montana Department of Fish, Wildlife and Parks, 3201 Spurgin Road, Missoula, MT 59801, USA

Moore, J. N., S. N. Luoma, and D. Peters. 1991. Downstream effects of mine effluent on an intermontane riparian system. Can. J. Fish. Aquat. Sci. 48: 222–232.

Metal concentrations were determined in benthic biota, fish livers, water, and fine-grained sediment through 215 km of an intermontane river system (Blackfoot River, Montana, USA) affected by headwater inputs of acid-mine effluent. Solute and particulate contaminants decreased rapidly downstream from headwater sources, but some extended through an extensive marsh system. Particulate contaminants penetrated through the marsh system, effectively resulting in food web contamination downstream of the marshes. Metals differed in their bioavailability within and below the marsh system. Cadmium was most consistently accumulated in the food web, and the general order of downstream mobilization of bioavailable metals appears to be Cd, Zn > Cu > As, Ni. Depauperate benthic communities and reduced fish populations occurred coincident with the sediment contamination.

Les concentrations de métaux ont été déterminées sur 215 km dans le benthos, des foies de poisson, l'eau et les sédiments fins d'un bassin hydrographique intramontagneux (rivière Blackfoot, Montana, É.-U.) touché par des effluents acides de mine déversés à la source de ce bassin. Les concentrations de contaminants en solution ou particulaires allaient en décroissant rapidement des eaux de tête vers l'aval, mais une certaine part de ces contaminants s'est répandue dans un important réseau de marais. Les contaminants particulaires ont pénétré dans le réseau de marais et ont contaminé le réseau alimentaire en aval des marais. La biodisponibilité des métaux variaient tant dans les marais qu'en aval de ces derniers. Le cadmium est le métal le plus abondamment retrouvé dans le réseau alimentaire; en aval, l'importance relative de la contamination effective par ces métaux biodisponibles suivait l'ordre suivant : Cd, Zn > Cu > As, Ni. La contamination des sédiments était corrélée à un appauvrissement des communautés benthiques et à une réduction des populations de poisson.

Received March 30, 1989
Accepted August 20, 1990
(JA115)

Reçu le 30 mars 1989
Accepté le 20 août 1990

As water supplies are stressed, there is concern over the effects and extent of metal contamination in the headwaters of drainage basins in western North America from past hard-rock mining activities (NOVA 1990). However, systematic studies of downstream sediment contamination and subsequent availability to biota of metals mobilized by acid-mine drainage are rare. Many studies of metal-contaminated riparian systems have focused on only a few kilometres of river near the input of contaminants (Moraiarity et al. 1982; Bradley and Cox 1987) and do not integrate contamination in the solid and aqueous phases with the biological system. As well, metal bioavailability in cobble bed rivers is poorly understood in general, partly because the biological communities of such systems are complex and difficult to sample. In this paper, we present data showing that headwater inputs of aqueous and particulate metal contaminants into a cobble bed river can affect chemical trends, metal concentrations, and metal bioavailability over a distance of tens of kilometres from the source. The goal is to understand the biological fate of metals mobilized by acid mine drainage, and specifically address the following questions: (1) Are high concentrations of metals near inputs of mine effluent available to resident biota? (2) Do beaver ponds/marsh systems (often characteristic of headwater streams) mitigate contaminant transfer downstream? (3) If metals are trapped in such a marsh system, do they remain available to resident biota? (4) How does spatial distribution of contamination in food webs compare with the particulate and aqueous phases of contamination? (5) Does contaminant bioavailability differ among metals or taxa? (6) Does metal bioavailability vary substantially from year to year?

Six taxa of benthic insect larvae, from two trophic levels, and three taxa of trout were sampled as representatives of the food web in a river system in western Montana (the Blackfoot River) contaminated by headwater inputs of mine effluents (acid mine drainage). Metal concentrations (Al, Cd, Cu, Fe, Pb, and Zn plus the metalloid As) were determined in whole bodies of benthic biota, fish livers, water, and fine-grained sediment. Metal contamination in benthic insects was compared with particulate and aqueous contamination, and contamination trends compared with distribution of fish populations in the

river. To assess the area of contaminant distribution, the complete river system was examined from the headwaters to its mouth, 215 km downstream, including inputs from all mine effluent sources. Comparisons were made with earlier studies conducted during 1971–75 (Spence 1975) to determine the temporal changes in the system.

Study Area

The Blackfoot River of western Montana is a Class I trout stream (Montana Department of Fish, Wildlife and Parks classification system) dissecting geologically complex, mineralized terrains of the northern Rocky Mountains. The river drains 6000 km^2, and from its source near the Continental Divide flows 215 km where it joins the Clark Fork River (Fig. 1). Mining in the headwaters of the Blackfoot River began in approximately 1865 and continued until after World War II. A variety of minerals and commodities were recovered including gold, silver, lead, and copper from numerous small placer and hard-rock mining operations. Although milling was not widespread in the drainage, milled tailings were discharged into the headwaters at several sites. The numerous small deposits of contaminated tailings and isolated sources of contaminated mine drainage in the upper reaches of the catchment (Fig. 1; Spence 1975) are typical of many montane areas in western North America.

The gradient of the Blackfoot River ranges from 0.5 to 60 m/km and is characterized by distinct changes in slope. The stair-step nature of the stream profile results from deposition of glacial deposits during the Pleistocene at the mouths of several tributaries, producing low-gradient reaches separated by higher gradient sections. Tributaries in the headwaters which drain the mining areas have relatively high gradients. Downstream from the mined areas, the river valley has been partially dammed by Pleistocene glacial till and outwash to form several low-gradient reaches. One such reach, extending from 211.6 to 196.6 km, has three extensive marshes (river kilometres are measured from the mouth upstream).

Earlier studies (Spence 1975) showed that acid-mine drainage caused enrichment in sulfate and metals (including Al, Cd, Ca, Cu, Pb, Mn, and Zn) in Blackfoot water downstream to approximately 190 km. Waters flowing from tunnels and bore holes were commonly of pH < 3 and no fish were found in tributaries affected by this acidic drainage (Spence 1975; Dollhopf et al. 1988). Drainage from adits and waste piles continue to supply contaminated water to the headwaters of the drainage. Spence found that bed sediment in the upper Blackfoot River was especially enriched with Cd, Cu, and Zn and that the bed was coated with Fe–Mn oxyhydroxide for several kilometres. This contamination is still visually and chemically evident today. In the early 1970's, marshes along the upper river appeared to impede downstream movement of the metals, as shown by reductions in contamination below the uppermost wetland (Spence 1975). Thus the stream morphology established a natural mitigation process that appeared to limit downstream dispersion of mine effluents.

The benthic community in much of the Blackfoot River is a diverse assemblage of insect larvae typical of temperate, cobble bed rivers (Spence 1975). The trout fishery is a complex, multispecies assemblage (Spence 1975; Peters and Spoon 1989). In general, eastern brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Oncorhynchus clarki lewisi*) are the dominant species from the headwaters to approximately 191.5 km. A transition zone occurs below 190 km to 168.5 km where brown trout (*Salmo trutta*) dominate the assemblage. Brown trout are replaced by rainbow trout (*Oncorhynchus mykiss*) in the lower river (below 85 km). Large, fluvial bull trout (*Salvelinus confluentus*) also are found in the middle and lower river. The trout fishery is an important recreational resource in western Montana.

Methods

Water Chemistry

Grab samples of river water were taken at 22 sites on the main stem, 9 on tributaries and 9 from contaminated tributaries

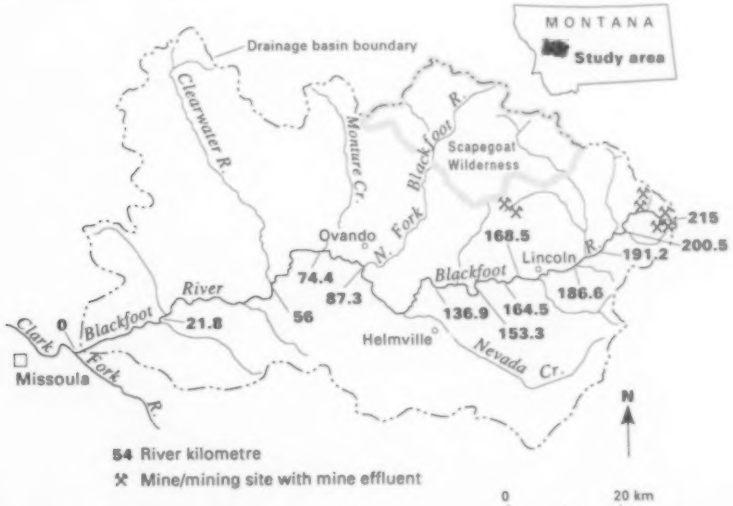


FIG. 1. Map of the Blackfoot River drainage basin, with biological sampling locations designated by kilometre from the mouth of the river.

(those tributaries with known mine effluent input). One sample from each location was filtered in the field through a 0.45-μm nucleopore membrane filter that was first rinsed with river water. The filtered sample was preserved for metal analyses by acidifying with concentrated, "instra-analyzed" nitric acid. Metal analyses were performed by inductively coupled argon plasma spectrometry (ICAPES). Detection limits are listed in Table 1. Another filtered sample was left unacidified for anion analysis by ion chromatography (IC). A third water sample was left unfiltered and unacidified for alkalinity measurement by titration (Gran method). All samples were transported to the laboratory on ice and refrigerated until analysis. Quality control during ICAPES analysis was maintained by using field and procedural blanks and U.S. Geological Survey water standards (Table 1). IC analyses were checked using internal standards.

Sediment Chemistry

Sediment samples were taken at 43 sites on the main stem, tributaries, and contaminated tributaries. Eight sites were replicated in 1988 and 1989 to determine annual variation (which was within the error of measurement). Surficial bed sediment was collected by suction in a large syringe (a turkey baster) from small deposits of fine-grained sediment over an area of 50 to 100 m^2 and composited by sieving through a 63-μm nylon screen. The resulting slurry was stored on ice, transported to the laboratory, centrifuged, and dried at 70°C. Dried sediment (0.2 g) was dissolved in a microwave digestion vessel in 5 mL of aqua regia and 2 mL of HF. After microwaving for 5 min at high power, the sample was filtered and the HF neutralized with boric acid. Then the sample was diluted 500:1 (v/w) and analyzed by ICAPES. Quality control was evaluated by using NBS sediment standards (Table 1).

Benthic Insect Larvae

Samples of benthic insect larvae were collected for metal analysis from 13 different stations in 1987, 1988, and 1989. Four stations were sampled between 190 and 17 km in 1987 and eight stations through that reach in 1988. Stations between 211.8 and 186.6 km (including 190 km) were sampled from riffles within and below the marsh system in 1989. The six taxa targeted for collection were relatively abundant, larger insect larvae from three functional groups. These included two predaceous Plecoptera (stoneflies), *Claassenia sabulosa* and *Hesperoperla pacifica* (family Perlidae), and four Trichoptera (caddisflies), the net-spinners *Hydropsyche* sp. and *Arctopsyche grandis* (family Hydropsychidae), the herbivore *Brachycentrus* sp. (family Brachycentridae), and the large case-bearing scavenger *Limnophilus* sp. *Brachycentrus, Limnophilus,* and *Hydropsyche* samples were not separated to species. Metal concentrations among species of *Hydropsyche* do not differ greatly in other systems (Cain et al. 1989).

The larvae were collected by kick net from rifle areas. For reference, metal concentrations from the Blackfoot taxa were compared with animals from Rock Creek, an undisturbed stream located in an adjacent drainage (Luoma et al. 1989; Axtmann et al. 1990). Taxa were separated for analysis and brachycentrids and limnophilids were removed from their cases in the field. Living animals were held for 4 to 6 h in bags of ambient water after separation for cleansing of their digestive tracts before the samples were frozen. In preparation for analyzes, frozen samples were thawed, the length of individuals from the larger taxa (*Claassenia* and *Hesperoperla*) was determined, and similar sized individuals were composited to a dry weight of at least 50 mg, but not more than 1.5 g. Microscopic verification of field taxonomy was conducted where necessary

TABLE 1. Concentrations (and standard deviations) for U.S. Geological Survey (USGS) water standards, National Bureau of Standards (NBS) sediment standard, and NBS bovine liver standard. Limits of detection (LOD) listed for solution only. All values listed in parts per million (ppm) for solids and mg/L for solutions, except where in weight % which is identified by an asterisk. NC = noncertified value; BD = below detection limit.

		Water standards				Sediment standard NBS 1645		Bovine liver NBS 1577a	
		USGS T103		USGS T97					
Metal	LOD	Given	Obtained ($n=6$)	Given	Obtained ($n=6$)	Given	Obtained ($n=4$)	Given	Obtained ($n=6$)
Al	0.03	0.127 (0.038)	0.136 (0.006)	0.126 (0.042)	0.156 (0.007)	2.26 (0.04)*	2.29 (0.09)*	2 (NC)	3.02 (0.51)
As	0.05	0.032 (0.0009)	BD	0.0113 (0.0015)	BD	66 (NC)	63 (8.4)	0.047 (0.006)	BD
Cd	0.005	0.0017 (0.0004)	0.002 (0.001)	0.0163 (0.0023)	0.016 (0.002)	10.2 (1.5)	6.7 (0.5)	0.44 (0.06)	0.37 (0.02)
Cu	0.005	0.0833 (0.0057)	0.082 (0.005)	0.0168 (0.0025)	0.015 (0.003)	109 (19)	101 (2.3)	158 (7)	155 (0.98)
Fe	0.005	0.041 (0.0076)	0.039 (0.002)	0.100 (0.009)	0.105 (0.004)	11.3 (1.2)*	9.16 (0.28)*	194 (20)	187 (3.5)
Mn	0.005	0.009 (0.0021)	0.007 (0.001)	0.0305 (0.0032)	0.029 (0.001)	785 (97)	695 (15)	9.9 (0.8)	9.95 (0.11)
Pb	0.04	0.0077 (0.0021)	BD	0.015 (0.0037)	BD	714 (28)	614 (16)	0.135 (0.015)	BD
Zn	0.005	0.0265 (0.0041)	0.033 (0.002)	0.153 (0.01)	0.159 (0.006)	1720 (170)	1525 (37)	123 (8)	127 (0.6)

on frozen samples. Each composite was digested by concentrated nitric acid reflux, the concentrated acid was evaporated, and the sample was reconstituted in 0.5 N HCl. Metal analyses in 1987 were conducted by flame atomic absorption spectrophotometry. Analyses in 1988 and 1989 were conducted by ICAPES.

The goal of benthic insect larvae collection at each site was to obtain at least 20 individuals of each available taxon with sufficient mass of each to exceed analytical detection limits. Achieving these goals for a variety of taxa in cobble bed rivers is made difficult by the spatial, temporal, and size variability typical of the benthic community (Giesey et al. 1981; Selby et al. 1985). As a result, more individuals of some taxa were collected than others at each station, the number of composites that represented an individual taxon at a site varied, and not all taxa were represented at all stations. Where more than one composite of a taxon was collected, confidence intervals were calculated to illustrate within-site variability. Data from adjacent sites also were aggregated in some cases to eliminate pseudoreplication and to test if differences between segments of the river were statistically different. Differences between segments or stations were tested by one-way ANOVA or by *t*-test. Data were log-transformed for statistical comparisons.

Fish

Fish were collected for metal analyses in 1988 and 1989. The metals of principal concern in the Blackfoot (Cd, Cu, and Zn) typically concentrate strongly in the liver (Bollingberg and Johansen 1979; Wiener and Giesy 1979). Thus, analyses of this organ were employed to determine the distribution of metal contamination of the Blackfoot fishery, as proven effective elsewhere (Roch et al. 1982). All comparisons of metal concentrations were restricted to similar size classes of fish within a species (Brooks et al. 1976; Chernoff and Dooley 1979). To compare contamination in the upper Blackfoot with a reference, cutthroat trout also were collected from Marshall Creek, a small stream undisturbed by mining near the Blackfoot River, but not in the drainage (not on Fig. 1). All fish were collected by electrofishing from river reaches exceeding 1 km long and immediately placed on ice. The length of each individual fish was measured and the liver removed for analysis within 6 h of collection. Liver samples were digested by the concentrated nitric acid reflux method described above and analyzed by ICAPES. NBS standard 1577a (bovine liver) was analyzed by the same method (Table 1). All statistical comparisons employed one-way ANOVA with log-transformed data.

Fish populations were estimated with the mark and recapture method, employing Chapman's modification of the Peterson formula described by Ricker (1975). Variance estimates were made with Chapman's formula (Ricker 1975). These methods were employed to provide direct comparison with a similar approach employed in earlier fish surveys in the Blackfoot (Spence 1975). Similar habitat was sampled from all sections that were compared. Detailed methods and a complete discussion of the fish population survey are available in Peters and Spoon (1989). Data from all biological and sediment metal analyses are available in Moore (1990).

Results

Solute Contaminants

The water in the lower main stem of the river (below 190 km) and in uncontaminated tributaries is a Ca–Mg–HCO_3 type low

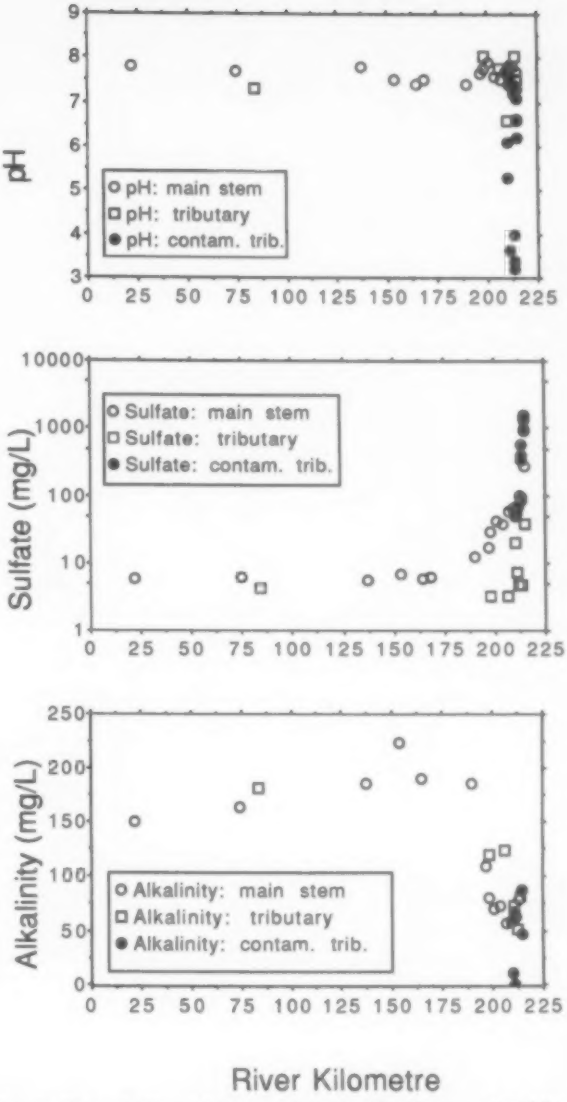


FIG. 2. Plots of pH, sulfate, and alkalinity ($CaCO_3$) vs. river kilometre. Tributaries and contaminated tributaries are plotted at the kilometre where they join the main stem of the Backfoot River. Contaminated tributaries have known mine effluent inputs; tributaries have no known mining inputs.

in sulfate, nitrate, fluoride, and trace constituents with pH's from 7.4 to 8.0. Contaminant input from mine effluent in the headwaters into the Blackfoot River is apparent from downstream trends of solute constituents. The pH of contaminated tributaries that flow into the upper 5 km of the river (mostly above the upper marsh system, but two contaminated tributaries flow directly into the upper marsh) ranges from 3 to 6.5 (Fig. 2). Concentrations of sulfate are extremely high in the acidic tributaries (Fig. 2) and decrease downstream away from input.

Both acidity and sulfate concentration show downstream attenuation from the headwater sources in the Blackfoot River. Acidity is neutralized rapidly and pH reaches background (uncontaminated tributary and downstream mainstem) values

before reaching the marsh system (211.6 km). Sulfate, however, shows less attenuation and penetrates downstream through the marshes; values above background extend to 190 km. Alkalinity of some mine effluent is near zero, but alkalinity of main stem and tributary water ranges from 50 to 100 mg $CaCO_3$ (including contaminated tributaries). Alkalinity generally increases downstream (Fig. 2).

Trends of Cd, Cu, and Pb in water show minimal injection of contaminants downstream from the sources (Fig. 3; As was below detection limit, 0.050 mg/L, for all samples exept in two mine effluents, as was Ni; other elements analyzed are not plotted). Concentrations of these elements decrease to values not detectably different from uncontaminated tributaries (mostly below detection limit) within the upper few kilometres of river. Iron, Mn, and Zn show more intrusion downstream (Fig. 3), but nearly all solute components reach detection limits 25 km downstream. Only Zn shows irregularities from this trend (possibly resulting from sample contamination).

These trends show that although most solute trace metals (Al, Cd, Cu, Mn, Ni, and Pb) are elevated from one to three orders of magnitude levels in the contaminated tributaries and the upper few kilometres of river, they decrease to levels of detection before entering the marsh systems at 211.5. Sulfate, Fe, Mn, and Zn are detectable farther downstream, into and through the upper marsh.

Total effluent input, from all adits and tunnels (all above 210 km) during low flows, accounts for approximately 7 to 14% of total river flow at 207 km (Spence 1975). Although there are several sources for the contaminants, one adit supplies most of the flow into the headwaters. Approximately 80% of

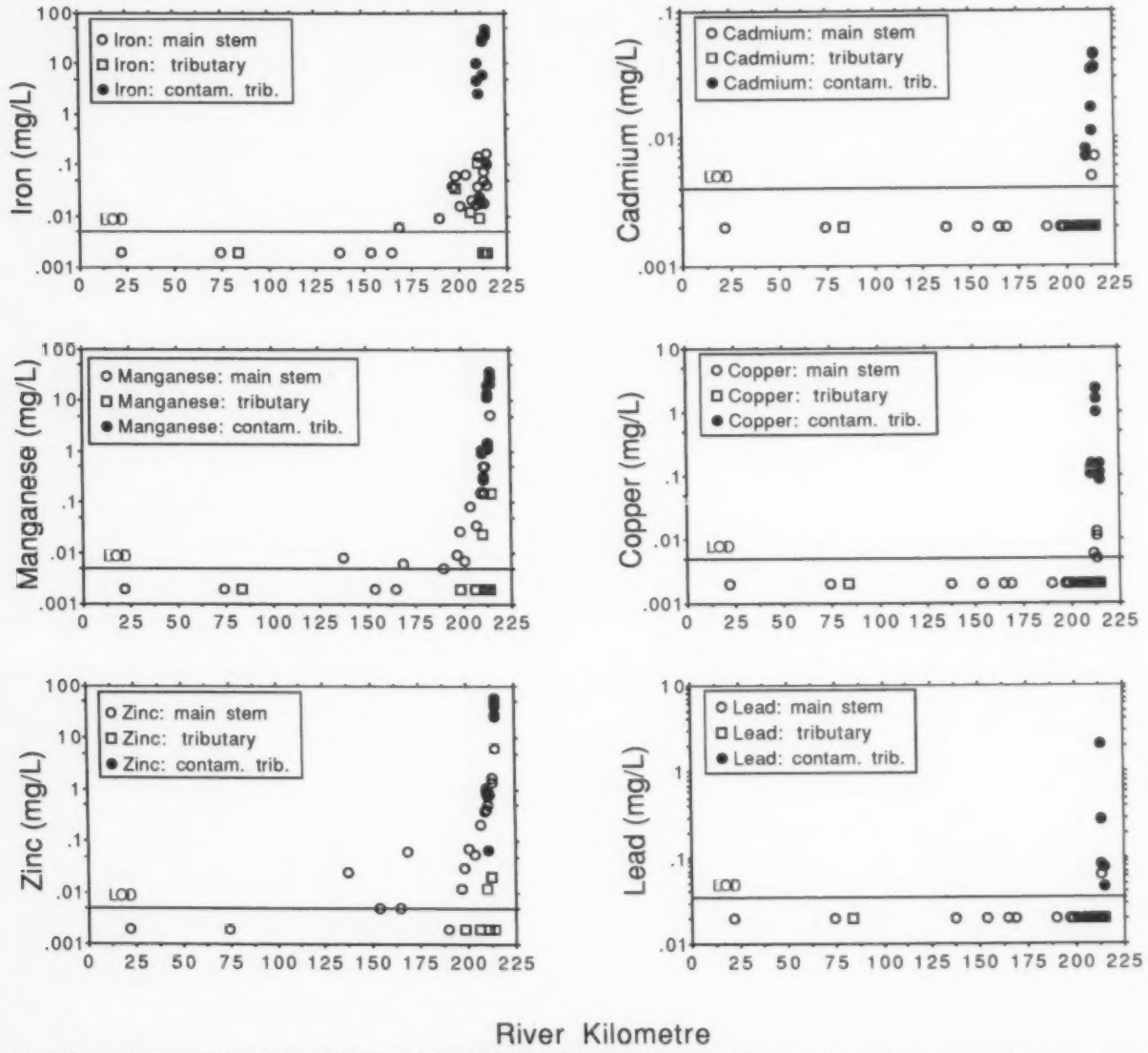


FIG. 3. Concentrations of Fe, Mn, Zn, Cd, Cu, and Pb in Blackfoot River water vs. river kilometre. Tributaries and contaminated tributaries are plotted at the kilometre where they join the main stem of the Blackfoot River. Contaminated tributaries have known mine effluent inputs; tributaries have no known mining inputs.

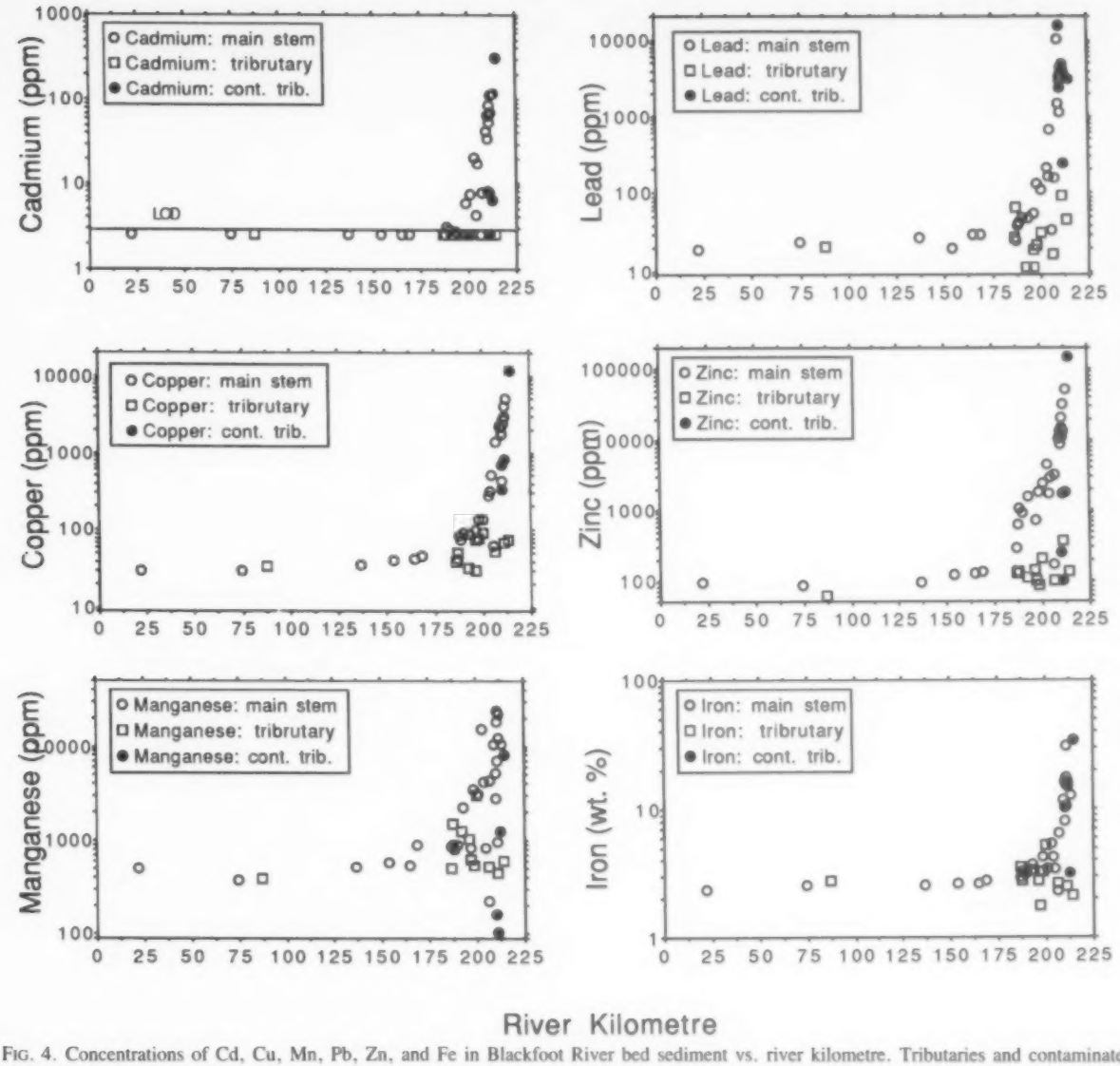


FIG. 4. Concentrations of Cd, Cu, Mn, Pb, Zn, and Fe in Blackfoot River bed sediment vs. river kilometre. Tributaries and contaminated tributaries are plotted at the kilometre where they join the main stem of the Blackfoot River. Contaminated tributaries have known mine effluent inputs; tributaries have no known mining inputs.

the mine effluent comes from that one mine at the farthest upstream (215.1 km) input (Spence 1975). These inputs are possibly diluted at high flows but no measurements have been made on mine inputs during spring runoff.

Sediment Contaminants

Compositional characteristics are distinctly different and concentrations of the trace elements As, Cd, Cu, Mn, Ni, Pb, and Zn are 10 to 1000 times higher in bed sediments in the upper few kilometres of the Blackfoot River compared with uncontaminated tributaries (background values) or sediment downstream (Fig. 4; As is only high in the uppermost reaches of the river and Ni is less enriched than all other metals, so these are not plotted). Solute and sediment contamination have similar sources. The sediment contamination originates from a small number of headwater tributaries and the major input of mine effluent at 215.1 km in the headwater mainstem. Concentrations of metals in sediments decrease rapidly downstream. It is unclear from these trends how much of the decrease is the result of downstream dilution of contaminants by uncontaminated sediment from tributaries, and how much is due to the action of the marshes. Trends through the marshes were complex and details differed from metal to metal. Figure 5 demonstrates that sediments 10 km beyond the marsh system (at 190 km) were substantially contaminated with metals such as Cu and Zn, compared with farther downstream.

Iron follows the trend of the trace elements (Fig. 4), higher-than-background concentrations penetrating downstream approximately 25 km from the main sources. In contrast, Al,

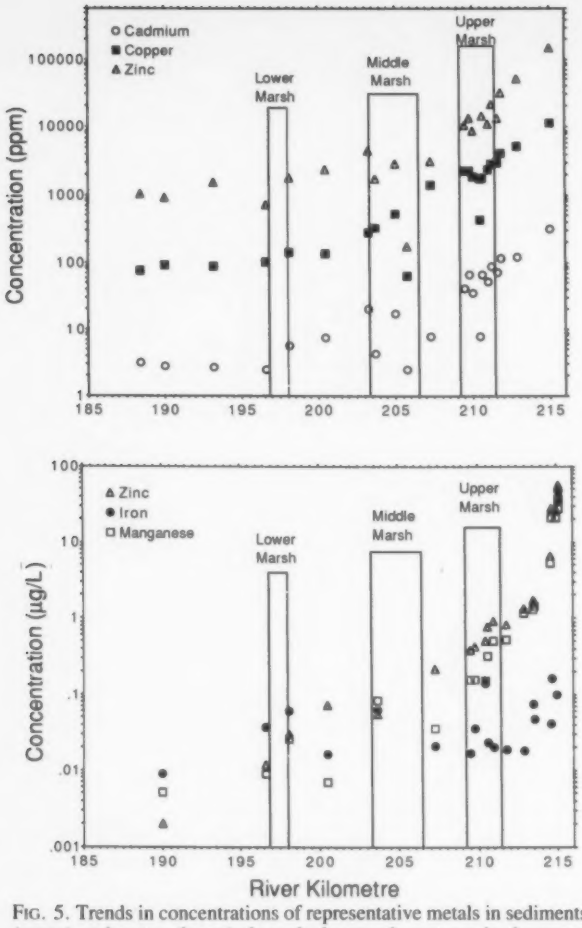


FIG. 5. Trends in concentrations of representative metals in sediments (upper) and water (lower) through the marsh systems in the upper Blackfoot River. Blocks indicate the locations of marshes.

Ca, and Ti were depleted in the headwaters and then increased rapidly downstream to values similar to most tributary and downstream sediments (data in Moore 1990).

In the upper few kilometres of river it is visually apparent that contaminated sediment is associated with Fe–Mn oxyhydroxides. All contaminated tributaries and the upper main stem have beds coated with red–orange oxyhydroxide precipitates. As acid mine drainage flows from the subsurface into the oxygenated surface waters, oxyhydroxides precipitate from solution, along with other hydroxide and sulfate minerals (Nordstrom 1982). These oxyhydroxides act as a source for particulate contamination downstream (Jones 1986; Filipek et al. 1987; Nordstrom 1982).

Metals in Biota

A qualitative estimation of presence/absence of targeted benthic fauna and mayflies (a metal-sensitive order, Winner et al. 1980) indicated that important faunal groups were absent where sediment was most contaminated. No benthic biota were found at 211.8 km where rocks were obviously coated with Fe–Mn oxyhydroxides. Limnophilids were the only targeted benthos found at 207 km. The communities at 101 and 190 km also were missing taxa typical of such streams. For example, hydropsychid caddisflies and mayflies were absent at 200.5 km in 1989. Absent groups at 190 km included hydropsychid caddisflies in 1988 and 1989, perlid stoneflies in 1988, and mayflies at least in 1989 (the only year the search included mayflies). Hydropsychids, perlids, and mayflies were present at 186.6 km and all other downstream stations.

TABLE 2. Year-to-year differences of Cd concentrations (µg/g dry weight) at three stations in the Blackfoot River in two types of insect larvae and cutthroat trout. Caddisfly is *Arctopsyche grandis*. Data for *Hesperoperla pacifica* and *Claassenia sabulosa* are combined to obtain stonefly comparison. NC = not collected; NP = not present.

Kilometre	Year	Caddisfly	Stonefly	Cutthroat trout
21.8	1987	<0.2	0.12	
	1988	0.30	0.10	
	1989	0.16	0.08	
74.4	1987	0.20	0.20	
	1987	0.18	0.09	
190	1987	NC	1.30	NC
	1988	3.27	NP	0.61 ± 0.21
	1989	1.26	0.76	0.60 ± 0.08

Downstream trends in metal concentrations in the food web of the river were similar in the three surveys. Year-to-year variability at three stations are exemplified for Cd in three taxa in Table 2 (all data in Moore 1990). Because of the general agreement among years, data from 1987, 1988, and 1989 were aggregated to summarize spatial distributions in the following analysis.

Metals differed from one another in their bioavailability within and below the marsh system. Cadmium was the element most consistently accumulated throughout the food web. It was significantly enriched ($p < 0.01$) in caddisflies, stoneflies, and brook trout collected from within the marsh system, when compared with downstream segments of the river (Tables 3 and 4). Although only two cutthroat trout were found in the marsh collections, their livers contained concentrations of Cd more than 30 times higher than found at the reference site. Significant Cd enrichment also appeared throughout the food web at 190 km, downstream from the marsh sytem, suggesting that the Cd contamination in sediments that penetrated through the marshes was bioavailable. Cadmium concentrations in caddisflies, stoneflies, and both species of trout were significantly higher at 190 km than in the middle (137 to 168 km) and lower (17 to 87 km) segments of the river (Tables 3 and 4). Cutthroat trout contained 3.3 times more Cd at 190 km than in the reference area ($p < 0.01$).

Bioavailable Cd contamination appeared to extend to the middle reaches of the river. Cadmium concentrations in caddisflies were not significantly different between the middle and lower segments of the river, as observed in sediments (Table 3). However, in stoneflies and in large brown trout, significant ($p < 0.05$ for insects; $p < 0.01$ for brown trout) Cd contamination was observed in the middle reach compared with the lower reach (Tables 3 and 5). The longer life and/or predaceous nature of these two groups appeared to make them sensitive indicators of Cd contamination.

Significant ($p < 0.01$) Zn contamination of the food web also extended downstream through the middle reach of the river as observed in *Brachycentrus*, *Arctopsyche*, and *Hesperoperla* (see Moore 1990 for data). Concentrations of Zn in brook trout livers were enriched significantly within, but not below, the

TABLE 3. Spatial distribution of Cd (μg/g dry weight ± 1 standard deviation) in insect larvae in the Blackfoot River. Reference values are from Rock Creek, a tributary of the Clark Fork River unaffected by mine effluent. NC = not collected; NP = not present.

Kilometre	*Limnophilus*	*Brachycentrus*	*Arctopsyche*	*Hesperoperla*	*Claassenia*
211.8	NP	NP	NP	NP	NP
Beginning of masches					
207.3	1.8	NP	NP	NP	NP
200.5	NP	13.8	NP	0.64 ± 0.16	0.69 ± 0.30
End of masches					
190	0.56 ± 0.14	2.3 ± 1.4	2.6 ± 0.08	1.04 ± 0.62	0.52 ± 0.17
186.6	0.42	0.54 ± 0.07	0.42	1.00	
168–137	NP	0.37 ± 0.21	0.07	0.27 ± 0.12	0.24 ± 0.04
74–87	NP	0.32 ± 0.11	0.14	0.14 ± 0.14	0.13 ± 0.06
Reference	NP	NC	0.10 ± 0.01		

TABLE 4. Cadmium, Cu, and Zn concentrations (μg/g dry weight ± 1 standard deviation) in the livers of trout from the upper Blackfoot River and a reference (Marshal Creek). All animals were 67 to 225 cm in length. The number of animals analyzed in one to six composites is shown in parentheses. NP = not present.

	Cd		Cu		Zn	
Kilometre	Brook	Cutthroat	Brook	Cutthroat	Brook	Cutthroat
211.8	18 ± 13(6)	NP	1113 ± 351(6)	NP	654 ± 68(6)	NP
Beginning of marshes						
207.3	9.4 ± 2.4 (25)	8.8 (2)	311 ± 51(25)	24(2)	324 ± 58(25)	135(2)
200.5	4.7 ± 1.5(9)	5.9(3)	182 ± 89(9)	34 ± 18(3)	235 ± 115(9)	146(3)
End of marshes						
190	0.70 ± 0.26(7)	0.60 ± 0.08(17)	135 ± 57(7)	14 ± 5(17)	138 ± 12(7)	107 ± 3(18)
186.6	<0.05(1)	0.17 ± 0.06(16)	54(1)	13 ± 5(16)	151(1)	106 ± 1(16)
Reference	NP	0.18 ± 0.07(18)	NP	15 ± 6(18)	NP	103 ± 3(18)

TABLE 5. Comparison of metal concentrations in livers of large brown trout (*Salmo trutta*), 341 to 475 cm in length, sampled in the middle reach of the Blackfoot River (near 123 and 134 km) with trout sampled below the confluence of the North Fork of the Blackfoot River at 74 km. * Significantly different from one another.

Kilometre	*n*	Cd	Cu	Zn
123–134	12	0.83 ± 0.42*	846 ± 398	111 ± 10
74	6	0.07 ± 0.05*	494 ± 223	111 ± 9

marsh system (Table 4). No significant differences in Zn concentrations occurred among stations in *Limnophilus* or *Claassenia*. The latter species may regulate Zn concentrations within their tissues.

Of the targeted species in this study, only brook trout (a depauperate population) were found upstream of the marsh system. Metal concentrations in the livers of the animals in this population suggested that substantial bioavailability of Cd, Zn, and Cu existed in conjunction with the high concentrations in the sediments and water. Metal concentrations in sediment and brook trout correlated strongly throughout the river, partly because of these upstream coincidences ($r^2 = 0.971$ for Cu, 0.922 for Zn, and 0.938 for log Cd). Zinc in brook trout was also correlated with Zn in water ($r^2 = 0.984$). Brook trout was the taxon in which the most obvious Cu contamination was observed (Table 4). The Cu concentration in the one brachycentrid composite from within the marsh was significantly ($p < 0.05$), but only slightly, higher than observed at 190 km. No statistically significant differences occurred among any segments of the river below the marshes ($p > 0.05$) in insect larvae, cutthroat trout, or brown trout (Tables 4 and 6). Such results suggest that Cu was either of less widespread availability in the Blackfoot River than were Cd or Zn, or Cu was physiologically regulated by most of the species studied. Arsenic and Ni were somewhat variable, but neither element showed consistent enrichment in any species in any segment of the river. Both appeared to be of low bioavailability (data in Moore 1990).

Historic Comparisons of Fish Populations

Trout populations were recently studied in the Blackfoot River (Peters and Spoon 1989) because of an increase in complaints about dwindling numbers of fish. Selected data from that study, relevant to the determinations of metal distributions, are presented here and compared with surveys from the 1970's. The most striking results are from upstream. Eastern brook trout populations declined between 1971–73 and 1988 at three of four stations between 170 and 200 km; cutthroat trout populations declined at two of three relevant stations in this reach. Data from 200 km illustrate the magnitude of those changes and the differences in response between species and age classes (Fig. 6A and 6B).

The surveys of Spence (1975) were conducted first in 1973 and then again in 1975 following the failure of an abandoned tailings pond in the uppermost headwaters of the river (input at 214.6 km just below the largest source of mine effluent). "Young-of-the-year" brook trout (age 0) were present in 1973 at 200 km, but absent in 1975, suggesting acute mortalities

TABLE 6. Spatial distribution of Cu (μg/g dry weight ± 1 standard deviation) in insect larvae in the Blackfoot River. Reference values are from Rock Creek, a tributary of the Clark Fork River unaffected by mine affluent. NC = not collected; NP = not present.

Kilometre	*Limnophilus*	*Brachycentrus*	*Arctopsyche*	*Hesperoperla*	*Claassenia*
211.8	NP	NP	NP	NP	NP
Beginning of marshes					
207.3	27	NP	NP	NP	NP
200.5	NP	28.9	NP	32 ± 1	33 ± 6
End of marshes					
190	15 ± 2	17 ± 4	18 ± 1	23 ± 6	41 ± 5
186.6	27	16 ± 1	10	28	
168–137	NP	19 ± 3	15 ± 1	24 ± 3	42 ± 10
74–87	NP	16 ± 1	14 ± 4	29 ± 3	43 ± 8
Reference	NP	NC	12 ± 4	21	

from mine/milling wastes injected into the river by the pond failure (Fig. 6A). In 1988, age 0 brook trout populations had returned, but the fish were only 33% as abundant as in 1973. Age 1 (1 yr old) and older brook trout showed no apparent mortality from the pond failure; population densities were the same in 1973 and 1975. However, the effects of poor recruitment that would be expected from the reduced abundance of age 0 fish were evident in 1988. Age 1 and older brook trout populations were 72% lower in 1988 than during 1973–75, declining in average density downstream from 82/km to 23/km.

Age 0 cutthroat trout at 200 km also showed severe acute mortality in 1975; age 1 and older cutthroat were reduced in abundance (compare 1973 and 1975 in Fig. 6B). Age 0 cutthroat populations in 1988 remained severely reduced compared with 1973. In 1988, the abundance of age 1 and older cutthroat trout was less than 25% of that in 1973 and had declined by an additional 50% from 1975 (an 85% decline in cutthroat compared with 1975 was observed upstream at 207 km in 1988).

Differences between surveys also occurred in brown trout populations downstream from the most severe contamination. For example, at 170 km, significantly fewer age 1 brown trout and a possible downward trend in 2-yr-old plus older brown trout were observed from 1972 to 1988 (Fig. 6C). However, unlike trout populations in metal-affected areas, no decline in age 0 brown trout was evident.

Discussion

In the Blackfoot River, some solute and particulate contaminants extend downstream for 25 km from the major contaminant source, while others contaminate only a few kilometres of river immediately below inputs. Concentration of solute contaminants decreases rapidly in the upper few kilometres of input, probably related to the precipitation of Fe oxyhydroxides. As metal-rich mine effluent cascades down steep reaches of river, the entrainment of oxygen precipitates solute Fe and Mn (Michaelis 1988). The high concentration of sulfate also leads to the precipitation of metal sulfates (Nordstrom 1982). Other metals and metalloids can coprecipitate with or adsorb onto these sulfates and oxyhydroxides. This system of solute contaminant precipitation or adsorption works well in decreasing solute metal contaminants in the Blackfoot drainage above the marsh sytem but transfers metals to the particulate phase. Some solute and particulate contamination passes through the marsh system, and at least Cd and Zn remain bioavailable over long stretches of the river.

Within the marsh and downstream, Cd contamination is more severe than Zn and affected more species.Arsenic and Ni originating from acid mine drainage are of low bioavailability consistent with their relatively low concentrations in both the solute and particulate phases. Elevated solute Cu concentrations decrease to near limits of detection before entering the marsh system, which may partially explain their apparent limited availability to the biota. Although Cu is elevated in brook trout, the particulate Cu contamination that extends through the marsh system has no detectable effects on concentrations in other representatives from the food web.

Longer lived, predaceous species appeared to be more sensitive indicators of downstream Cd contamination than detritus feeding species and they were also better indicators than sediments or water. Significant Cd contamination was observed in stoneflies and large brown trout more than 75 km downstream from the input of acid mine drainage. Zinc was also significantly accumulated in some species in the middle reaches of the river. Thus, the order of downstream mobilization of bioavailable metals originating from the acid mine drainage appears to be Cd, Zn > Cu > As, Ni.

It is well established that natural marshes can improve water quality (Wolverton 1987) and that aquatic plants and bacteria can remove metals from acid mine drainage (Wolverton 1975; Wolverton and McDonald 1977). Natural and artificial marshes can decrease acidity, sulfate, and metal solute concentrations in acid mine drainage by 1 to 2 orders of magnitude (Huntsman et al. 1978; Wieder et al. 1982) by precipitation, adsorption, hydrolysis, redox and photochemical reactions, chelation, and filtration. The period of time over which marshes provide effective mitigation and the pathways that transfer contaminants from the mine effluent to the biota are poorly known. The Blackfoot River is typical of Rocky Mountain rivers contamined by abandoned mine drainage with a well-established beaver pond/marsh system that has received mine effluent for more than 100 yr. The Blackfoot example suggests that marsh systems may slow the transport of metals, but they do not necessarily completely stop downstream contamination of the food web by the most mobile of the metals. Particulate contaminants generally penetrated the marsh system to a greater extent than did solute contamination, but the marsh system is complex geochemically, possibly acting as both sink and source for different particulate metal contaminants.

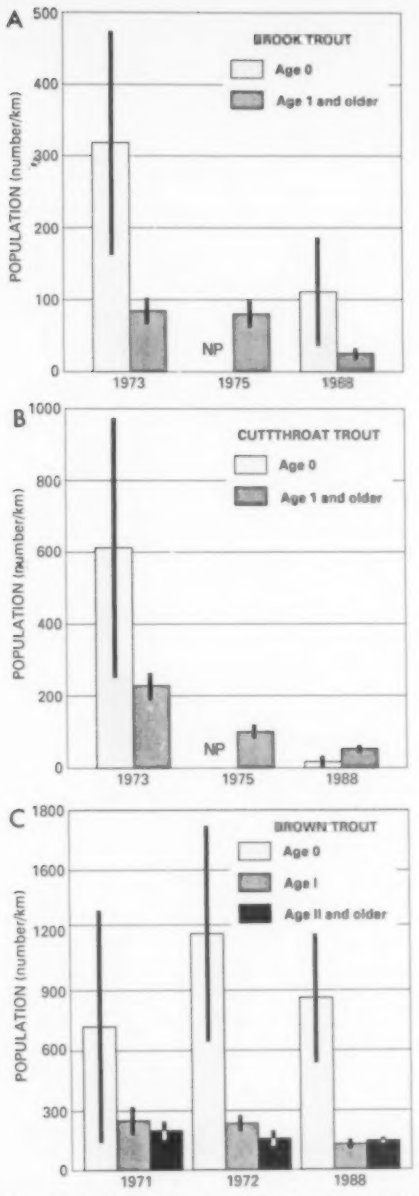


FIG. 6. Plots of population density of age 0 and age 1 plus older (A) brook trout and (B) cutthroat trout at 200 km in the Blackfoot River compared between the 1970's and 1988. (C) Brown trout population density at 170 km compared between the two sampling periods.

Other studies have shown that metals such as Cd, Pb, and Zn adversely affect both trout and their food web (Duncan and Klaverkamp 1982; Niederlehner et al. 1984; Hendrix et al. 1982; Selby et al. 1985; Harrison and Klaverkamp 1989; Schmitt and Finger 1987; Schmitt et al. 1984), although manifestation of such effects can be complex (Munkittrick and Dixon 1988; Schindler 1987). Adverse ecological effects of metals cannot be discounted in the Blackfoot River, where substantial contamination of sediment, water, and food webs from acid mine drainage is accompanied by depauperate benthic communities and fish populations. Surveys of the Blackfoot River conducted during low flow during 1968–71 (Spence 1975) also indicated a reduced number of bottom fauna species, especially mayflies, at 206.6 and 198.1 km but not at 192.6 or 186.5 km. The present study detected sediment contamination at 190 km and an absence of typical insect larvae (e.g. mayflies). This is a minimum data set, but it suggests that metal influences may extend further downstream than observed 16 yr earlier. Such changes may result from single large events or progressive change over decades, but are not detectable in consecutive years of sampling.

Before natural or artificial marshes are considered as long-term solutions for remedying continuous trace metal inputs from acid mine drainage, our study of the Blackfoot River suggests that a thorough study of particulate, aqueous, and biological components and consideration of long-term changes is necessary. If such systems typically fail to mitigate effects of upstream inputs of metals for an indefinite period, elimination of sources of metal input in such systems may be the most effective mechanism for preventing extended, long-term riverine contamination in Rocky Mountain riparian systems.

Acknowledgements

This work could not have been accomplished without the continuing contribution of ideas and enthusiasm from D. Cain and E. Axtmann plus the able field assistance of E. Axtmann, D. Cain, M. Cook, S. Andrews, V. Elrod, and D. Pridmore. Carolyn Johns originally suggested the idea of an integrated sediment–water–biota study on the Blackfoot and supplied laboratory and editorial assistance.

References

AXTMANN, E. V., D. J. CAIN, AND S. N. LUOMA. 1990. Distributions of trace metals in fine-grained, bed sediments and benthic insects in the Clark Fork River, Montana: Proceedings, Clark Fork River Symposium, Missoula, MT, April 20, 1989.

BOLLINGBERG, H. J., AND P. JOHANSEN. 1979. Lead in spotted wolfish, *Anarhichas minor*, near a zinc–lead mine in Greenland. J. Fish. Res. Board Can. 36: 1023–1028.

BRADLEY, S. B., AND J. J. COX. 1987. Heavy metals in the Hamps and Manifold Valleys, North Staffordshire, U.K.: partitioning of metals in floodplain soils. Sci. Total Environ. 65: 135–153.

BROOKS, R. R., J. R. LEWIS, AND R. D. REEVES. 1976. Mercury and other heavy metals in trout of Central North Island, New Zealand. J. Mar. Freshwater Res. 10: 233–244.

CAIN, D. J., J. L. CARTER, AND S. V. FEND. 1989. Temporal and spatial variability of arsenic in benthic insects from Whitewood Creek, South Dakota, *In* G. E. Mallard and S. E. Ragone [ed.] U.S. Geological Survey Toxics Substances Hydrology Program — Proceedings of the technical meeting, Phoenix, AZ, Sept. 26–30, 1988. U.S. Geol. Surv. Water Resour. Invest. Rep. 88–4220.

CHERNOFF, B., AND J. K. DOOLEY. 1979. Heavy metals in relation to the biology of the mummichog, *Fundulus heteroclitus*. J. Fish Biol. 14: 309–328.

DOLLHOPF, D. J., J. D. GOERING, R. B. RENNICK, R. B. MORTON, W. K. GAUGER, J. B. GUCKERT, P. M. JONES, K. C. COOKSEY, K. E. BUCKLIN, R. WEED, AND M. M. LEHMAN. 1988. Hydrochemical, vegetational and microbiological effects of a natural and a constructed wetland on the control of acid mine drainage. Montana Department of State Lands, Abandoned Mine Reclamation Bureau, Helena, MT, Reclamation Research Publication 88-04, June 1988. 214 p.

DUNCAN, D. A., AND J. F. KLAVERKAMP. 1982. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci. 40: 128–138.

FILIPEK, L. H., D. K. NORDSTROM, AND W. H. FICKLIN. 1987. Interaction of acid mine drainage with waters and sediments of west Squaw Creek in the West Shasta Mining district, California. Environ. Sci. Tech. 21: 388–396.

GIESEY, J. P., J. W. BOLLING, H. J. KANIA, R. L. KNIGHT, AND S. MASHBURN. 1981. Fates of cadmium introduced into channels microcosm. Environ. Int. 5: 159–175.

HARRISON, S. E., AND J. F. KLAVERKAMP. 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem. 8: 87–97.

HENDRIX, P. E., C. L. LANGNER, AND E. P. ODUM. 1982. Cadmium in aquatic microcosms: implications for screening the ecological effects of toxic substances. Environ. Manage. 6: 543–553.

HUNTSMAN, B. E., J. G. SOLCH, AND M. D. PORTER. 1978. Utilization of *Sphagnum* species dominated bog for coal acid mine drainage abatement. Geol. Soc. Am. Abstr. Program 10(7): 426.

JONES, K. C. 1986. The distribution and partitioning of silver and other heavy metals in sediments associated with an acid mine drainage stream. Environ. Pollut. 12B: 249–263.

LUOMA, S. N., E. V. AXTMANN, AND E. J. CAIN. 1989. Fate of mine wastes, Clark Fork River, Montana, U.S.A., p. 63–75. *In* Metals and metalloids in the hydrosphere; impact through mining and industry. Asian Inst. Tech. (UNESCO), Bangkok, Thailand.

MICHAELIS, H. V. 1988. Integrated biological systems for effluent treatment from mine and mill tailings, p. 99–112. *In* W. Solomons and U. Forstner [ed.] Environmental management of solid waste. Springer Verlag, New York, NY.

MOORE, J. N. 1990. Mine effluent effects on non-point source contaminants in the Blackfoot River, Montana. Report to the Montana Department of Health and Environmental Sciences, Water Quality Bureau, Helena, Montana, May 1990. 64 p.

MORAIARITY, F., K. R. BULL, H. M. HANSON, AND P. FREESTONE. 1982. The distribution of lead, zinc and cadmium in sediments of an ore-enriched lotic ecosystem, the River Ecclesbourne, Derbyshire. Environ. Polzut. 4B: 45–68.

MUNKITTRICK, K. R., AND D. G. DIXON. 1988. Growth, fecundity, and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45: 1355–1365.

NIEDERLEHNER, B. R., A. L. BUIKEMA, JR., C. A. PITTINGER, AND J. CAIRNS, JR. 1984. Effects of cadmium on the population growth of a benthic invertebrate *Aeolosoma headleyi* (Oligochaeta). Environ. Toxicol. Chem. 3: 255–262.

NORDSTROM, D. K. 1982. Aqueous pyrite oxidation and the consequent formation of secondary iron minerals. Soil Sci. Soc. Am. Spec. Publ. 10: 37–56.

NOVA. 1990. Poison in the Rockies (transcript of video). WGBH-TV, Boston, MA. p. 2–3.

PETERS, D., AND R. SPOON. 1989. Preliminary fisheries inventory of the Big Blackfoot River. Montana Department of Fish, Wildlife and Parks, Missoula, MT. 46 p.

RICKER, W. F. 1975. Handbook of computations for biological statistics of fish populations. Fish. Res. Board Can. Bull. 119: 300 p.

ROCH, M., J. A. MCCARTER, A. T. MATHESON, M. R. CLARK, AND R. W. OLAFSON. 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39: 1596–1601.

SCHINDLER, D. W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 6–25.

SCHMITT, C. J., F. J. DWYER, AND S. E. FINGER. 1984. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catastomidae). Can. J. Fish. Aquat. Sci. 41: 1030–1040.

SCHMITT, C. J., AND S. E. FINGER. 1987. Effects of sample preparation on measured concentrations of eight elements in edible tissues of fish from streams contaminated by Pb mining. Arch. Environ. Contam. Toxicol. 16: 185–207.

SELBY, D. A., J. M. IHNAT, AND J. J. MESSER. 1985. Effects of subacute cadmium exposure on a hardwater mountain stream microcosm. Water Res. 19: 646–655.

SPENCE, L. E. 1975. Upper Blackfoot River — A preliminary inventory of aquatic and wildlife resources. Montana Department of Fish and Game, Missoula, MT. 236 p.

WIEDER, R. K., G. E. LANG, AND A. E. WHITEHOUSE. 1982. Modification of acid mine drainage in a fresh water wetland, p. 38–62. *In* Proceedings, Acid Mine Drainage Research and Development, West Virginia Surface Mine Drainage Task Force Symposium, Charleston, WV.

WIENER, J. G., AND J. P. GIESY, JR. 1979. Concentrations of Cd, Cu, Mn, Pb, and Zn in fishes in a highly organic softwater pond. J. Fish. Res. Board Can. 36: 270–279.

WINNER, R. W., M. W. BOESEL, AND M. P. FARRELL. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can. J. Fish. Aquat. Sci. 37: 647–655.

WOLVERTON, B. C. 1975. Water hyacinths for removal of cadmium and nickel from polluted waters. NASA Tech. Memo. TM-X-72721.

1987. Artificial marshes for wastewater treatment, p. 141–152. *In* K. R. Reddy and W. H. Smith [ed.] Aquatic plants for water treatment and resource recovery. Magnolia Publ. Inc., Orlando, FL.

WOLVERTON, B. C., AND R. C. MCDONALD. 1977. Wastewater treatment utilizing water hyacinths (*Eichhornia crassipes*) (Mart) Solms, p. 205–208. *In* Treatment and disposal of industrial wastewaters and residues. Proceedings of National Conference, Houston, TX.

Spatial Variation in the Distribution of Phosphorus Species in the Surficial Sediments of Canadohta Lake, Pennsylvania: Implications for Internal Phosphorus Loading Estimates

M. L. Ostrofsky and G. G. McGee

Department of Biology, Allegheny College, Meadville, PA 16335, USA

Ostrofsky, M. L., and G. G. McGee. 1991. Spatial variation in the distribution of phosphorus species in the surficial sediments of Canadohta Lake, Pennsylvania: implications for internal phosphorus loading estimates. Can. J. Fish. Aquat. Sci. 48: 233–237.

The concentrations of total, loosely sorbed, metal-bound, calcium-bound, and organic phosphorus, iron, and organic matter (loss on ignition) were determined for 71 surface sediment samples from Canadohta Lake, Pennsylvania. The results indicate that lake sediments can be highly variable with respect to these parameters. Most of these parameters were highly intercorrelated, and some general patterns appear to exist based on these and previously published data. Given the highly variable nature of sediment quality, particularly among these parameters which have been shown to be good predictors of sediment phosphorus release rates, estimates of internal phosphorus loading based on a few incubated cores taken from the deepest area of a lake may contain serious errors. We recommend that sediment heterogeneity be assessed in order to determine an appropriate number and distribution of cores to be incubated for internal loading estimates.

Les concentrations de phosphore, de fer et de matière organique faiblement sorbés, liés à des métaux et à du calcium et leur concentration totale de même que celle du phosphore organique ont été déterminées (perte par calcination) dans 71 échantillons de sédiments de surface du lac Canadohta, en Pennsylvanie. Les résultats montrent que ces paramètres peuvent varier de façon très importantes dans les sédiments lacustres. La plupart de ces paramètres étaient fortement intercorrélés, et certaines relations régulières semblent exister selon les données présentées ici et d'autres déjà publiées. Étant donné que les caractéristiques des sédiments sont très variables, particulièrement les paramètres reconnus pour donner de bonnes indications sur les taux de libération du phosphore contenu dans les sédiments, les estimations de la charge interne de phosphore fondées sur l'analyse de seulement quelques carottes incubées recueillies dans les zones les plus profondes d'un lac peuvent donner des résultats très erronés. Nous recommandons d'évaluer l'hétérogénéité des sédiments, ce qui permet de déterminer le nombre et la distribution appropriés de carottes à échantillonner, pour obtenir une bonne estimation de la charge interne.

Received January 2, 1990
Accepted August 20, 1990
(JA428)

Reçu le 2 janvier 1990
Accepté le 20 août 1990

The importance of phosphorus supply in determining the trophic status of lakes is well established, and there exist several empirical models which allow limnologists and lake managers to predict the consequences of changes in phosphorus loading to lakes. It has been noted, however, that lakes with anoxic hypolimnia behave differently than do lakes with anoxic hypolimnia (Nürnberg 1984), particularly with respect to the fate of sedimented phosphorus. Lakes with anoxic hypolimnia commonly receive a significant portion of their phosphorus loads from the lake sediments, and this internal load of phosphorus is perhaps the reason why many of the empirical models available underestimate phosphorus concentration when applied to these lakes. Nürnberg (1984) presented two revised models which would allow a more accurate estimation of total phosphorus concentration in these lakes. The first replaces predicted phosphorus retention with measured retention in the basic Dillon and Rigler (1974) model. This suffers from the necessity of having to measure phosphorus retention, a measurement which requires a considerable committment to sampling and analysis. The second model adds the internal load corrected by the areal water load to the Dillon and Rigler (1974) model:

$$(1)\quad [P_{spr}] = L_{ext}(1 - R_{pred})/q_s + L_{int}/q_s.$$

In this model, phosphorus retention may be predicted from one of many empirical models in the literature (e.g. Kirchner and Dillon 1975; Larson and Mercier 1976; Ostrofsky 1978; Nürnberg 1984). This second model, however, assumes that the internal loading of phosphorus is known.

The internal phosphorus load to lakes has been estimated in a variety of ways, most commonly by measuring the increase in the mass of phosphorus either in the hypolimnion or in water overlying intact sediments in core tubes. The accuracy of the former approach is limited by the presence of sedimenting seston of epilimnetic origin, and by the possibility that some released phosphorus may be converted to particulate form by way of physical, chemical, or biological processes and resedimented. When release rates are estimated from intact sediment cores, this has most often been done on the basis of a few replicate cores taken from the deepest area of the lake (e.g. Nürnberg 1987). The internal phosphorus load to lakes may be estimated as the product of phosphorus release rate determined from changes in phosphorus mass in anoxic water overlying undisturbed sediment cores, the duration of anoxia in the lake, and the area of sediments overlain by anoxic water (Nürnberg 1984).

Estimates of release rates determined from incubated cores can be highly variable, however. In an analysis of release rates based on multiple cores from seven lakes Nürnberg (1987) provided data which indicated that the variance of release rate estimates can often equal or exceed the mean estimate. As a result, for any particular lake, one or only a few incubated cores will lead to an estimate for internal loading which may not be particularly accurate. While there may be a variety of factors leading to this variability, one factor which we wish to examine here is sediment heterogeneity. Ostrofsky et al. (1989) and Nürnberg (1988) have shown that much of the variation in phosphorus release rates may be explained by differences in sediment quality, specifically the distribution of sedimentary phosphorus species, iron, and organic matter. There is no reason to expect that a few cores from the deepest area of a lake will adequately describe either the nature of the lake's hypolimnetic sediments or give an accurate estimation of phosphorus release rates from the lake's sediments in general. We need to know how variable a lake's sediments are, and whether or not there is any pattern to this variability. With this knowledge in hand, we are then in a better position to design a sampling program which will allow us to collect and incubate the appropriate number of cores to estimate internal loading rates with some statistical accuracy.

The purpose of this study was to assess the variability of phosphorus species, iron, and organic matter (loss on ignition) in the surficial sediments of a small lake. Sediment heterogeneity is of considerable importance in its own right (Downing and Rath 1988). However, since these parameters have been found useful in predicting phosphorus release rates from anoxic sediments to the overlying hypolimnetic water mass, we were particularly interested in this variability as an analog for variability in phosphorus release rates.

Methods

This study was conducted on the sediments of Canadohta Lake, Crawford County, Pennsylvania (41°49′N, 79°50′W). The lake is small (68 ha) and has a maximum depth of about 13.6 m and a mean depth of 5.5 m. Its watershed (about 20 km^2) is mixed agricultural land and deciduous forest. The lake shore is extensively developed with summer cottages..

During winter ice cover, eight lakewide transects were established and sampling sites were located at 45.7-m (150-ft) intervals along these transects (Fig. 1). At each site, a hole was drilled for sampling, the depth was measured with an echosounder, and sediment was collected with a Wildco K-B corer. Cores were extruded in the field, and the surface 2.5 cm of sediment was retained for analysis. We assumed that this increment would be responsible for the bulk of the phosphorus mass released during anoxic periods. In all, 71 samples of surface sediment were obtained.

In the laboratory, the distribution of phosphorus species was determined using the sequential extraction technique of Hieltjes and Lijklema (1980). This method yields concentrations of loosely bound (NH_4Cl-P), iron plus aluminum-bound (NaOH-P), and calcium-bound (HCl-P) phosphorus.

Total phosphorus (TOT-P) and total iron (IRON) were measured on $HClO_4/HNO_3$-digested samples, and organic phosphorus (ORG-P) was estimated as the difference between total phosphorus and the sum of the inorganic fractions. All phosphorus species were determined using the heteropoly blue method (Strickland and Parsons 1968), and iron was determined by atomic absorption spectrophotometry. All extractions and analyses were done in triplicate, and the means used in the analyses are reported here. All concentrations are reported as micrograms phosphorus per gram oven-dried sediment. Loss on ignition is reported as a percentage and total iron as milligrams iron per gram oven-dried sediment.

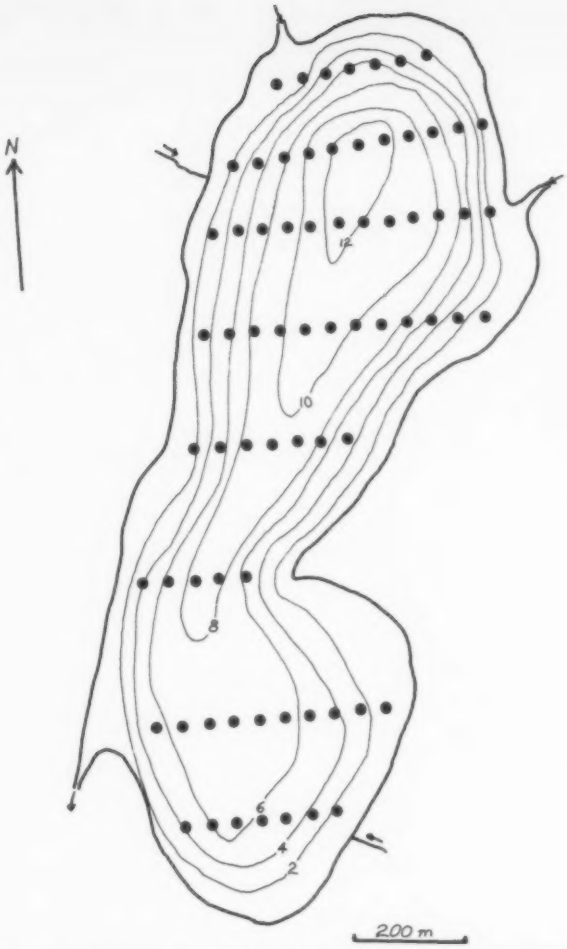


FIG. 1. Bathymetric map of Canadohta Lake showing sediment sampling locations.

Either natural log transformed or untransformed data were analyzed by regression. Those regression equations which explained the most variance in the dependent variable are reported here.

Results and Discussion

Canadohta Lake Data

Despite both the relatively small size of Canadohta Lake and the small size and geological uniformity of its catchment, values of most parameters were highly variable, with ranges spanning 1.5–2 orders of magnitude (Table 1).

Most of the parameters examined were strongly intercorrelated (Table 2). All phosphorus fractions were significantly related to total sedimentary phosphorus; regression equations were as follows:

TABLE 1. Descriptive statistics for the sediment characteristics measured on the 71 samples taken from Canadohta Lake. All phosphorus species in μg/g sediment dry weight, iron in mg/g sediment dry weight, and loss on ignition (LOI) in percent of dry weight.

Parameter	Mean	Range	Standard deviation	Coefficient of variation
NH_4Cl-P	29.2	1–65	16.2	55.5
NaOH-P	508.7	7–1019	241.5	47.5
HCl-P	149.7	37–261	40.0	26.7
ORG-P	767.1	44–1062	225.9	29.4
TOT-P	1454.9	267–2237	454.4	31.2
IRON	36.5	2.3–51.9	12.4	34.0
LOI	24.7	2–90	13.1	53.0

(2) $\ln(\text{ORG-P}) = 0.87 \ln(\text{TOP-P}) + 0.32, \quad r^2 = 0.88$

(3) $\ln(NH_4Cl\text{-P} = 1.75 \ln(\text{TOT0P}) - 9.47, \quad r^2 = 0.74$

(4) $\ln(\text{NaOH-P}) = 1.72 \ln(\text{TOT-P}) - 6.39, \quad r^2 = 0.80$

(5) $\ln(\text{HCl-P}) = 0.43 \ln(\text{TOT-P}) + 1.87, \quad r^2 = 0.34.$

Samples with increasing amounts of TOT-P tended to have relatively more NH_4Cl-P and NaOH-P (slopes of the regression lines significantly greater than 1; $p < 0.05$) and relatively less ($p < 0.05$) HCl-P. ORG-P was a relatively constant fraction of TOT-P (slope not significantly different from 1; $p > 0.05$). TOT-P and NaOH-P were significantly related to iron:

(6) $\ln(\text{TOT-P}) = 0.53 \ln(\text{IRON}) + 5.36, \quad r^2 = 0.61$

(7) $\ln(\text{NaOH-P}) = 0.89 \ln(\text{IRON}) + 2.92, \quad r^2 = 0.47$

and both TOT-P and IRON were significantly related to water depth (metres):

(8) $\text{TOT-P} = 550.08 \ln(\text{DEPTH}) + 526.31, \quad r^2 = 0.74$

(9) $\text{IRON} = 15.49 \ln(\text{DEPTH}) + 10.35, \quad r^2 = 0.79.$

Comparisons with Other Data Sets

There is little literature available to suggest answers to questions dealing with sediment heterogeneity, particularly with respect to sedimentary phosphorus species. There do exist two large data sets for comparisons, however. Williams et al. (1976) examined the distribution of phosphorus species in Lake Erie ($n = 48$) although they used an extraction sequence which yielded somewhat different fractions. Ostrofsky (1987) examined the distribution of phosphorus species in the profundal sediments of a broad range of lakes in eastern glaciated North America ($n = 66$). Means and variances of parameters measured in these studies (Williams et al. 1976, table 2; Ostrofsky 1987, table 3) may be compared with means and variances of the corresponding parameters from Canadohta Lake (Table 1). In both of these data sets, variability is relatively high, in the former because of the large size of the lake and the geological and land use heterogeneity of its catchment and in the latter because of the varied factors which make each lake somewhat unique.

Canadohta Lake sediments have significantly (t-test, $p < 0.05$) more TOT-P, ORG-P, and IRON than do Lake Erie sediments. Canadohta Lake sediments have significantly higher concentrations of NaOH-P than Lake Erie has NAI-P and significantly lower concentrations of HCl-P than Lake Erie has apatite-P. The relative importance of HCl-P/apatite-P in the sediments of Lake Erie may be a consequence of large erodable areas on both the north and south shores of the lake (Williams et al. 1976).

Despite the small size and geological and land use uniformity of Canadohta Lake's catchment, variability of the lake's sediments with respect to TOT-P and IRON were the same as the variability of Lake Erie sediments (variance ratio test, $p > 0.05$). Canadohta Lake sediments were more variable with respect to ORG-P, but less variable with respect to metal-bound phosphorus (NaOH-P versus NAI-P) and calcium-bound phosphorus (HCl-P versus apatite-P).

Comparing Canadohta Lake sediments with the profundal sediments of 66 lakes, Canadohta sediments had significantly lower concentrations, and lower variances of all measured parameters (TOT-P, ORG-P, NH_4CL-P, NaOH-P, HCl-P, IRON) except loss on ignition where mean and variance were both higher.

In each of the three data sets the sediment variables measured were highly intercorrelated, and in all cases, significant predictive relationships existed between ln (TOT-P) and the natural logs of the various phosphorus species, and between ln (IRON) and ln (NaOH-P) (or ln (NAI-P)) and ln (TOT-P). It is unknown whether these relationships are general ones or are unique to a particular lake or to the profundal regions only of lakes. Unfortunately, the Lake Erie data consist of phosphorus species which show only a general correspondence to the other two data sets, and those species which are equivalent among the data sets were analyzed using different methods. However, comparing the above relationships with those found for the 66 lakes where the same extraction sequence was used, there is evidence for some generality in some of the relationships. There is no difference between the slopes of the regression lines relating ln (TOT-P) and ln (NaOH-P), nor between those relating ln (IRON) and either ln (TOT-P) or ln (NaOH-P). In the regressions for ln (IRON) and ln (NaOH-P) there was no significant difference between intercepts, indicating that a single equation could be used to describe both data sets:

TABLE 2. Matrix of correlation coefficients among characteristics measured in 71 samples of Canadohta Lake sediment. Correlation coefficients greater than 0.23 and 0.30 are significant at $^*p < 0.05$ and $^{**}p < 0.01$, respectively.

	NaOH-P	HCl-P	ORG-P	TOT-P	IRON	LOI	Depth
NH_4Cl-P	0.59**	0.34**	0.66**	0.71**	0.66**	−0.21	0.52**
NaOH-P	—	0.28*	0.67**	0.91**	0.84**	−0.39**	0.92**
HCl-P		—	0.38**	0.44**	0.49**	−0.27*	0.22
ORG-P			—	0.91**	0.81**	−0.31**	0.55**
TOT-P				—	0.92**	−0.39**	0.80**
IRON					—	−0.49**	0.78**
LOI						—	−0.47**

(10) $\ln (\text{NaOH-P}) = 0.97 \ln (\text{IRON}) + 2.69, \quad r^2 = 0.45, n = 135.$

Slopes were different for the relationships between ln (TOT-P) and ln (NH_4Cl-P), ln (HCl-P), and ln (ORG-P).

From these analyses it might be speculated that the formation of iron–phosphorus complexes is the most consistent mechanism for phosphorus sedimentation. The slope of the above regression equation (10) is not significantly different from 1, suggesting that the amount of phosphorus sedimenting with iron is in constant proportion to the amount of iron. The remaining sedimented phosphorus is a function of such local or regional factors as the amount of detrital apatite derived from the catchment, or the relative proportions of phosphorus-rich autochthonous versus phosphorus-poor allochthonous organic matter.

Patterns with Depth

Boström et al. (1982) suggested that the concentration of total phosphorus in the sediments increases with water depth as a result of more or less continuous transport. Preliminary data from K. Dierolf and M. L. Ostrofsky (unpubl.) have shown a correlation between depth and the distribution of certain phosphorus species in Crystal and Conneaut lakes, Pennsylvania, and in this study we found significant relationships between depth and all measured parameters except HCl-P (Table 2). Reynoldson and Hamilton (1982), however, described the sediments of Lake Wabamun, Alberta, as being quite homogeneous. Depth information was not included in the data presented by Williams et al. (1976) but we estimated depths at their sampling locations by projecting their fig. 1a onto a bathymetric map of Lake Erie (NOAA chart No. 14820). Using these data we found no relationship between depth and the concentrations of any of their phosphorus species except organic phosphorus. Reynoldson and Hamilton (1982) attributed their observed sediment homogeneity to wind action, a factor of probable significance in a lake as broad and as shallow as Lake Erie. In lakes which are deep relative to their wind fetch, a more gentle sorting of sedimentary materials may be expected.

Implications for Loading Estimates

The implication of these observations is that at least some lakes show a great deal of variability with respect to those sediment characteristics which have been shown to be good predictors of phosphorus release. Specifically, Canadohta Lake has higher concentrations of iron, iron-bound phosphorus, and total phosphorus in deepwater sediments than in shallow-water sediments. This distribution would suggest that the phosphorus release rates from deeper sediments would be greater than the release rates from shallow-water sediments. In addition, deepwater sediments are exposed to the anoxic conditions which stimulate phosphorus release for a longer period of time. As a result of these two factors, whole-lake release rates estimated from cores taken only from the deepest area of such lakes are likely to be gross overestimates of actual internal loading rates. Actual internal loading rates might be calculated as the sum of the products of the release rates of sediments between successive depth contours, the surface area of sediments between these contours, and the duration of anoxia at these depths:

$$\text{Internal load} = \sum_{i=0}^{Z_{max}} A_i \times \text{RR}_i \times t_i,$$

where A is the area (square metres) of the ith contour, RR is the rate of release of phosphorus (milligrams phosphorus per square metre per day), and t is the duration (days) of anoxia.

In attempting to estimate internal loading of phosphorus from incubated sediment cores, the heterogeneity of a lake's sediments must be assessed in order to determine the number and distribution of samples required to adequately estimate phosphorus release rates. In lakes with relatively homogeneous sediments, a few cores may be sufficient. In lakes with highly variable sediments, like Canadohta, many cores might be necessary from each of a series of depth contours to make an adequate assessment of whole-lake phosphorus release rate. Nürnberg (1987, tables 2 and 3) provided some data to answer the question of how many cores might be required. Using the mean release rate and the standard deviation derived from SE and n for the three lakes (Gravenhurst Bay, Pt-10, and St. George) where a number of cores were collected from the same location, and presumably have the same sediment characteristics, it can readily be estimated how many cores would be necessary to have any desired level of confidence that the true release rate lies within any desired interval about the calculated mean. For example, if it were adequate to know that the true release rate is bounded by our estimated mean release rate plus and minus 25% of this mean with 95% confidence, it would be necessary to incubate 7, 13, and 45 cores from the three lakes, respectively. The mean release rate from these incubated cores would only tell us about the rate of release of phosphorus from sediments with similar characteristics, and another set of cores would be required to estimate release rates from sediments which were qualitatively different. In a lake such as Canadohta where it is probable that the deepwater sediments release more phosphorus, due not only to higher concentrations of phosphorus species which are highly correlated with release rates but also due to a longer interval of anoxia, it would be particularly important to have a reliable estimate of release rates from the deeper sediments. Sediments in shallower waters have a slower release rate and release phosphorus for a much shorter interval, so considerably more uncertainty may be tolerated here. Nevertheless, even in lakes where sediment quality is a predictable function of depth, considerable effort might be required to assess sediment heterogeneity and to estimate expected variance in replicate incubated cores prior to designing any sampling program to reliably estimate internal loading.

Acknowledgements

We are grateful to Mr. Robert Lizotte for assistance with the field sampling and to Ms. Darla Boyer-Locke for assistance in the laboratory.

References

Boström, B., M. Jansson, and C. Forsberg. 1982. Phosphorus release from lake sediments. Arch. Hydrobiol. Beih. Ergebn. Limnol. 18: 5–59.

Dillon, P. J., and F. H. Rigler. 1974. A test of a simple nutrient budget model predicting phosphorus concentration in lake water. J. Fish. Res. Board Can. 31: 1771–1778.

Downing, J. A., and L. C. Rath. 1988. Spatial patchiness in the lacustrine sedimentary environment. Limnol. Oceanogr. 33: 447–458.

Hieltjes, A. H. M., and L. Lijklema. 1980. Fractionation of inorganic phosphates in calcareous sediments. J. Environ. Qual. 9: 405–407.

Kirchner, W. B., and P. J. Dillon. 1975. An empirical method of estimating the retention of phosphorus in lakes. Water Resour. Res. 11: 181–182.

Larson, D. P., and H. T. Mercier. 1976. Phosphorus retention capacity of lakes. J. Fish. Res. Board Can. 33: 1742–1750.

Nürnberg, G. K. 1984. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. 29: 111–124.

1987. A comparison of internal phosphorus loads in lakes with anoxic hypolimnia: laboratory incubations versus in situ hypolimnetic phosphorus accumulation. Limnol. Oceanogr. 32: 1160–1164.

1988. Prediction of phosphorus release rates from total and reductant-soluble phosphorus of anoxic lake sediments. Can. J. Fish. Aquat. Sci. 45: 453–462.

OSTROFSKY, M. L. 1978. Modification of phosphorus retention models for use with lakes with low areal water loading. J. Fish. Res. Board Can. 35: 1532–1536.

1987. Phosphorus species in the surficial sediments of lakes of eastern North America. Can. J. Fish. Aquat. Sci. 44: 960–966.

OSTROFSKY, M. L., D. A. OSBORNE, AND T. J. ZEBULSKE. 1989. Relationship between anaerobic sediment phosphorus release rates and sedimentary phosphorus species. Can. J. Fish. Aquat. Sci. 46: 416–419.

REYNOLDSON, T. B., AND H. R. HAMILTON. 1982. Spatial heterogeneity in whole lake sediments — towards a loading estimate. Hydrobiologia 91: 235–240.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167.

WILLIAMS, J. D. H., J.-M. JAQUET, AND R. L. THOMAS. 1976. Forms of phosphorus in the surficial sediments of Lake Erie. J. Fish. Res. Board Can. 33: 413–429.

Arginine Vasotocin, an Osmoregulatory Hormone, as a Potential Indicator of Acid Stress in Fish

A. Hontela

Département des sciences biologiques, TOXEN, Université du Québec à Montréal, C.P. 8888, Montréal, Qué. H3C 3P8, Canada

J. B. Rasmussen

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montréal, Qué. H3A 1B1, Canada

D. Ko and K. Lederis

Department of Pharmacology, Faculty of Medicine, University of Calgary, Calgary, Alta. T2N 1N4, Canada

and G. Chevalier

Département des sciences biologiques, TOXEN, Université du Québec à Montréal, C.P. 8888, Montréal, Qué. H3C 3P8, Canada

Hontela, A., J. B. Rasmussen, D. Ko, K. Lederis, and G. Chevalier. 1991. Arginine vasotocin, an osmoregulatory hormone, as a potential indicator of acid stress in fish. Can. J. Fish. Aquat. Sci. 48: 238–242.

Brook trout (*Salvelinus fontinalis*) exposed for 1 wk to pH 5.6 (sublethal acidity) exhibited higher forebrain and pituitary arginine vasotocin (AVT) levels and lower plasma Na^+ levels than fish exposed to pH 6.7 (control). Addition of 300 μg total Al/L to water at pH 5.6 did not augment the effect on either the plasma Na^+ or the AVT levels. Exposure to demineralized water for 1 wk lowered the plasma Na^+ and increased AVT in the forebrain but had no effect on pituitary AVT. The increased AVT levels could be used as early warning indicators of sublethal acid stress in the brook trout. Such indicators, used in wild fish, might become important tools in management of trout lakes in areas threatened by acidification.

Des ombles de fontaine (*Salvelinus fontinalis*) exposées pendant 1 sem à un milieu de pH 5,6 (acidité sublétale) ont présenté des teneurs plus élevées en arginine-vasotocine (AVT) dans le cerveau antérieur et l'hypophyse et des teneurs moins élevées de Na^+ dans le plasma que des truites exposées à un milieu de pH 6,7 (témoin). L'ajout de 300 μg d'Al total/L au milieu de pH 5,6 n'a pas accru les teneurs en AVT et en Na^+ du plasma. L'exposition à de l'eau déminéralisée pendant 1 sem a entraîné une baisse de la teneur en Na^+ du plasma et une augmentation de la teneur en AVT du cerveau antérieur, mais n'a eu aucune incidence sur la teneur en AVT de l'hypophyse. Les plus importantes teneurs en AVT pourraient être utilisées comme un premier indicateur d'un stress d'acidité sublétale chez l'omble de fontaine. Un tel indicateur utilisé en milieu naturel pourrait devenir un important outil de gestion des lacs à truite dans les régions menacées d'acidification.

Received January 25, 1990
Accepted August 20, 1990
(JA448)

Reçu le 25 janvier 1990
Accepté le 20 août 1990

Populations of wildlife are subjected to a wide array of pollutants that may not cause mortality. While toxicology has relied greatly on LC50 to assess toxicity of pollutants, there is a need for bioindicators of "health" of organisms in their natural habitats (Heath 1987), indicators sensitive and specific enough to detect sublethal effects of a particular environmental stressor.

Soils in the Canadian Shield are poorly buffered and lakes at around pH 5.5 and 100–300 μg Al/L are common (Kelso et al. 1986). Although these conditions are not lethal to the more acid-resistant salmonids such as brook trout (*Salvelinus fontinalis*) (Daye and Garside 1975), reduced survivorship and/or reproductive impairment (Schofield and Trojnar 1980; Frenette and Dobson 1984; Cleveland et al. 1986), reduced growth rate (Rodgers 1984) as well as histopathological changes in the gills (Chevalier et al. 1985), and ionoregulatory disturbances (McDonald and Milligan 1988; Wood et al. 1988) have been reported under these conditions. Since acid and aluminum toxicity in salmonids involves ionoregulatory failure (>30% loss of blood Na^+ and Cl^- is lethal, Wood 1987), early warning indicators of ionoregulatory disturbance may be useful in the assessment of the health of fish exposed to sublethal acid and aluminum concentrations.

Osmoregulatory hormones, mediators of the ionoregulatory homeostasis, control fluxes of water and ions across the gills and other regulatory surfaces. Increased prolactin secretion during acid exposure has been reported in tilapia (*Sarotherodon mossambicus*); administration of prolactin to acid-stressed fish raised concentrations of plasma ions and blood osmolality and decreased water permeability of the gills (Wendelaar Bonga et al. 1983, 1988). The method for rapid measurement of prolactin concentrations in fish is currently being developed (Hirano et al. 1985). The effects of sublethal pH and aluminum on the concentrations of urotensins, osmoregulatory hormones of the caudal neurosecretory system (Lederis 1984), have been recently investigated in the brook trout (Chevalier et al. 1986; Hontela et al. 1989). Urotensin I levels measured by a quick and reproducible radioimmunoassay were highly correlated

with acidity of the water, while urotensin II levels were correlated with the concentrations of total aluminum in the water.

In this study, we investigated the potential of another osmoregulatory hormone, arginine vasotocin (AVT) (Sawyer 1977), as an indicator of sublethal acid stress in the brook trout. Fish were subjected to pH 5.6 with or without aluminum (300 μg/L), and after 1 wk, AVT concentrations in the forebrain, where AVT is synthesized, and in the pituitary, where AVT is stored prior to release, were measured by radioimmunoassay (Hontela and Lederis 1985) along with plasma Na^+ levels. In another experiment, fish were exposed to demineralized water, a treatment known to cause loss of plasma Na^+ in fish (Olivereau et al. 1982), to test the sensitivity of AVT secretion to ion loss.

Methods

Experimental Treatments

Sexually immature male and female brook trout (11.2 ± 0.7 g) were purchased from a hatchery (Des Landes de St. Félix de Kingsey, Qué.) in November. Upon arrival, fish were kept for 3–6 wk in flow-through holding stock tanks supplied with aerated and filtered well water at 14°C. A glass roof permitted maintenance of natural photoperiod (Montréal, Qué.) Fish were fed Purina Trout Chow once daily ad libitum.

Exposure to low pH and aluminum

Following the 2–3 wk in the stock tanks, feeding was stopped and fish were transferred to the experimental aquaria described previously by Hontela et al. 1989 (80 L, photoperiod 12 h light/12 h dark, 10°C, aerated artificial soft water at pH 6.7, Ca^{2+} = 0.1 meq/L, see Table 1) for 7 d. Then, one group was subjected to artificial soft water at pH 5.6, another group to pH 5.6 with 300 μg Al/L added, and a third group was kept at pH 6.7. After 7 d, fish were lightly anaesthesized with unbuffered MS 222 (tricaine methanesulphonate, 125 mg/L) and sampled.

Exposure to demineralized water

In the experimental aquaria, fish were first subjected to aerated artificial soft water for 7 d. Then, one group of fish was subjected to demineralized water (Table 1), while another group was kept in artificial soft water (all groups at pH 6.7 and 15°C). After 7 d, fish were anaesthesized and sampled.

Preparation of the experimental media

The artificial soft water was prepared by adding 12 mg of $NaHCO_3$, 7.5 mg of $CaSO_4 \cdot 2H_2O$, 7.5 mg of $MgSO_4$, and 0.5 mg of KCl to 1 L of demineralized water. The pH was adjusted with H_2SO_4, water aged for a minimum of 18 h, and pH rechecked and adjusted again if necessary. To prepare the reconstituted water with aluminum, powdered aluminum was dissolved in HCl to make up a stock solution which was added to artificial soft water. To ensure constancy of the physicochemical characteristics of the water in the tanks, 50% of the water was replaced daily. Water was drained from the tanks with a small hose attached to a pump and fresh water was added from the top to minimize disturbance of the fish. The pH was measured twice each day; the daily drift in pH did not exceed 0.2 unit in any of the aquaria and the other physicochemical parameters did not vary greatly within the experimental period (Table 1).

Sampling

Fish were sampled always at the same time of day, since daily fluctuations in AVT levels have been detected previously (Hontela and Lederis 1985). A blood sample was withdrawn from the caudal vasculature with a 1-cc heparinized syringe (Heparin, ammonium salt, Sigma), the blood was centrifuged, and plasma stored at −27°C for subsequent assessments of plasma Na^+ by flame emission spectrophotometer. The pituitary gland and the forebrain were dissected and washed in acetone, dried, weighed, and extracted. To extract the peptide hormone, the tissues were homogenized in 0.25% acetic acid, heated to 100°C for 3 min, and the supernatants were collected after centrifugation at 27 000 rpm and stored at −27°C.

Radioimmunoassay for AVT

The concentrations of AVT were determined in pituitary and brain extracts by radioimmunoassay as described previously (Hontela and Lederis 1985). A standard curve (Fig. 1) was established by making known amounts of the hormone compete against hormone labelled with ^{125}I for binding sites on rabbit antibodies against pure, synthetic AVT. The bound and the unbound hormones were then separated by a second antibody complex and the radioactivity was counted. Hormone levels in the extracts were determined from the standard curve. The differences between means were analyzed with a Student *t*-test at $p \leq 0.01$ and $p \leq 0.05$ levels.

Results and Discussion

This is the first study to investigate the potential of AVT as an indicator of acid stress in the brook trout. Physiological func-

TABLE 1. Chemical characteristics (mean ± SE) of water. An asterisk indicates a single measurement.

Treatment	pH	Al (μg/L)	Ca^{2+} (mg/L)	Conductivity (μS/cm)	Dissolved oxygen (mg/L)	Hardness (mg/L)
Stock	6.6 ± 0.06 (n = 14)	<10 (n = 4)	1.9*	25 ± 0.3 (n = 7)	11.2 ± 0.4 (n = 7)	11.5 ± 0.05 (n = 14)
pH 6.7	6.77 ± 0.16 (n = 14)	<10 (n = 4)	2.24*	22.0 ± 0.5 (n = 7)	11.3 ± 0.2 (n = 7)	8.9*
pH 5.6	5.59 ± 0.04 (n = 14)	<10 (n = 4)	2.18*	28.1 ± 0.1 (n = 7)	11.05 ± 0.3 (n = 7)	8.8*
pH 5.6 with Al	5.65 ± 0.05 (n = 14)	290 ± 24 (n = 4)	2.52*	24.5 ± 0.5 (n = 7)	11.39 ± 0.2 (n = 7)	9.8*
Demineralized water	6.51 ± 0.05 (n = 14)	<10 (n = 4)	<0.1	1.13 ± 0.04 (n = 7)	10.5 ± 0.04 (n = 7)	<1

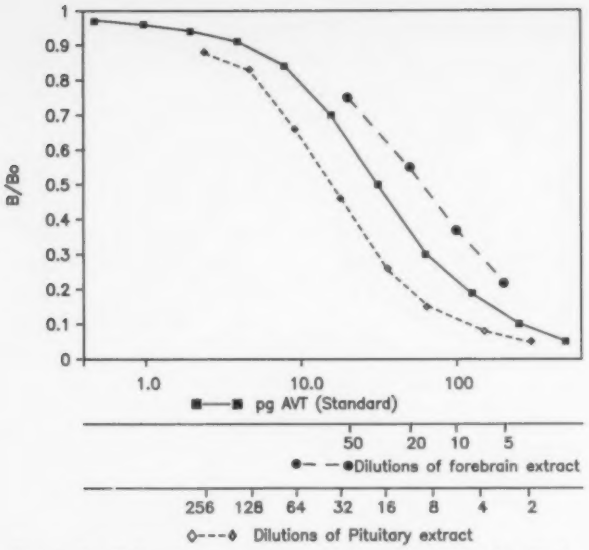


FIG. 1. Parallelism between a standard curve for AVT and a dilution curve of brook trout forebrain and pituitary extract. B/Bo = binding over maximum binding.

tions of AVT include localized vasoconstriction in the gills tending to redirect blood flow through the lamellae (Bennett and Rankin 1986), effects on elimination of water through kidneys (Maetz et al. 1964; Sawyer et al. 1982), and reduction of water entry through the gills (Lahlou and Giordan 1970). The net effect of AVT in a freshwater teleost fish seems to be one of countering the dilution of the internal milieu (Maetz et al. 1964). These effects of AVT on ion and water balance may be important during the physiological responses to acid/aluminum, treatments known to alter ion fluxes, ventilation rate and gas exchange across the gills (Malte 1986; McDonald and Milligan 1988; Walker et al. 1988), ionic balance of the internal milieu (Wood 1987; Audet et al. 1988), and gill morphology (Chevalier et al. 1985; Evans et al. 1988). Thus, we expected levels of this hormone to be altered by exposures to sublethal pH and aluminum treatment.

Effect of Low pH and Aluminum

The AVT levels in the brook trout forebrain were significantly higher at pH 5.6 than at pH 6.7 (Fig. 2), but aluminum did not exacerbate the effects of the sublethal pH on forebrain AVT. A similar pattern was observed for pituitary AVT levels. The 1-wk sublethal pH exposure resulted in a small (15%) but statistically significant loss of plasma Na^+, similar to that reported by others (Wood et al. 1988). The increase in forebrain and pituitary AVT concentrations which accompanied the decrease in plasma Na^+ has not been described previously. Several authors have reported a toxic effect of aluminum at pH 5.0–5.5 (Muniz and Leivestad 1980; Cleveland et al. 1986), but reports of greatly reduced toxic action of aluminum at pH above 5.5 have been reported as well (Sadler and Lynam 1987). The chemistry and toxicity of aluminum in acid water is complex (Neville and Campbell 1988), and although total aluminum in the water was determined in our study, the availability of toxic forms of aluminum was not assessed.

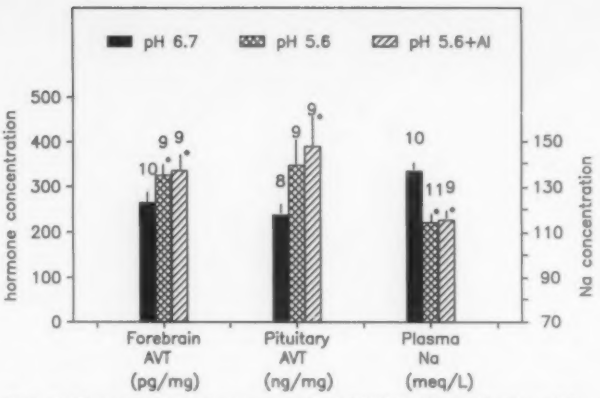


FIG. 2. Concentrations (mean ± SE) of AVT in the forebrain and in the pituitary and concentrations of plasma Na^+ in fish subjected for 1 wk to artificial soft water at pH 6.7 (control), pH 5.6, or pH 5.6 with 300 μg total Al/L (significant difference from control indicated by asterisks).

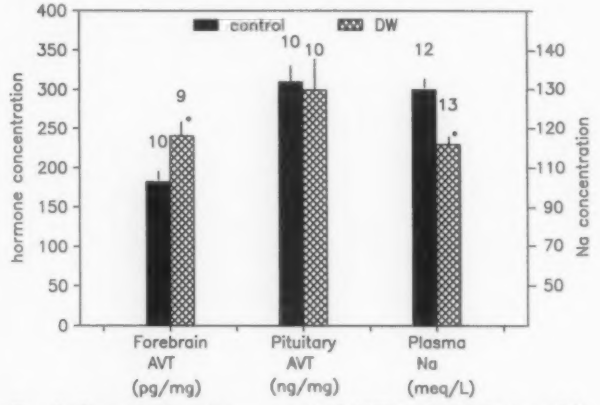


FIG. 3. Concentrations (mean ± SE) of AVT in the forebrain and in the pituitary and concentrations of plasma Na^+ in fish subjected for 1 wk to artificial soft water at pH 6.7 (control) or demineralized water (DW) at pH 6.7 (significant difference from control indicated by asterisks).

Effect of Demineralized Water

To study the relationship between AVT concentrations and plasma Na^+, AVT levels were determined in fish kept in demineralized water to lower their plasma Na^+ concentrations (Olivereau et al. 1982) and in fish kept in artificial soft water. Plasma Na^+ decreased and forebrain AVT levels increased (Fig. 3), but pituitary AVT levels were not influenced by the 1-wk treatment. Fluctuations in the quantity of neurosecretory material within the hypothalamo–pituitary axis of teleosts subjected to demineralized water were reported in earlier studies (Szabo and Molnar 1965; Olivereau 1967). The loss of plasma Na^+ may be the physiological signal initiating the changes in AVT secretion. Our data indicate that while the changes in forebrain AVT levels can be detected within 1 wk of treatment, a longer period of exposure to demineralized water may be required before pituitary levels of AVT change. In the pituitary, AVT is stored at levels three orders of magnitude higher than in the brain where AVT is produced.

The effect of the increased forebrain and pituitary AVT concentrations on availability of the hormone at the target organs

remains to be investigated. Measuring plasma AVT levels is impractical at the present time, since concentrations of AVT measurable in the plasma are at the lower limit of the sensitivity of the radioimmunoassay (5 pg/mL plasma, A. Hontela and K. Lederis, unpublished). In general, blood levels of hormones fluctutate on a shorter time scale than levels in the endocrine tissues where the hormones are synthesized or stored. It has been suggested that where endocrine parameters are used to assess the effect of a stressor, plasma and tissue endocrine responses be used in evaluation of acute and chronic stress, respectively (Donaldson et al. 1984). The forebrain and pituitary AVT levels in the brook trout were raised after 1 wk of exposure to the experimental condition; however, the dynamics of the changes in AVT levels have not yet been determined.

While our laboratory experiments suggest that AVT has potential as an indicator of acid stress in the brook trout, several characteristics of the AVT response remain to be investigated in order to establish the validity of this hormone as an acid stress indicator in field populations. The effect of the length of exposure to acid/aluminum on the AVT response is being tested in the laboratory in order to establish the temporal dynamics of the AVT response. In addition, we are measuring AVT in brook trout from acid and neutral lakes to determine AVT levels in fish chronically exposed to natural pH/aluminum conditions. The effects of season, sex, and size are of particular interest. We are also investigating the effects of handling on the AVT response. The forebrain and pituitary AVT levels in wild brook trout remain stable throughout the collection period and do not appear to be influenced by the method of capture. Experiments to test the effect of anaesthesia (MS 222) and stunning on AVT levels have not yet been carried out in the brook trout; however, a previous study on laboratory goldfish (A. Hontela et al., unpublished) showed similar AVT levels in fish sampled after anaesthesia with MS 222 and in fish stunned and sampled without MS 222.

Endocrine changes reflect homeostatic efforts of fish challenged by their environment, in this instance stressors, such as acidity or metals. The technique of measuring the concentrations of hormones by radioimmunoassay is quick and reproducible and is routinely used in clinical diagnosis. Measurements of osmoregulatory hormones may be useful in detection of sublethal acid and aluminum stress in fish and in the assessment of health of fish in acidified waters.

Acknowledgements

This project was supported by a MRC postdoctoral fellowship to A. H. and NSERC grant No. 9847 to G. C.; K. L. is a MRC career investigator. We thank Laurent Gauthier for his technical help and Yves Roy for making his expertise in the use of the aquatic facilities available to us.

References

AUDET, C., R. S. MUNGER, AND C. M. WOOD. 1988. Long-term sublethal acid exposure in rainbow trout (*Salmo gairdneri*) in soft water: effects on ion exchanges and blood chemistry. Can. J. Fish. Aquat. Sci. 45: 1387–1398.

BENNETT, M. B., AND J. C. RANKIN. 1986. The effects of neurohypophysial hormones on the vascular resistance of the isolated perfused gill of the european eel, *Anguilla anguilla* L. Gen. Comp. Endocrinol. 64: 60–66.

CHEVALIER, G., L. GAUTHIER, R. LIN, R. S. NISHIOKA, AND H. A. BERN. 1986. Effect of chronic exposure to an acidified environment on the urophysis of the brook trout, *Salvelinus fontinalis*. Exp. Biol. 45: 291–299.

CHEVALIER, G., L. GAUTHIER, AND G. MOREAU. 1985. Histopathological and electron microscopic studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. Can. J. Zool. 63: 2062–2070.

CLEVELAND, L., E. E. LITTLE, S. J. HAMILTON, D. R. BUCKLER, AND J. B. HUNN. 1986. Interactive toxicity of aluminum and acidity to early life stages of brook trout. Trans. Am. Fish. Soc. 115: 610–620.

DAYE, P. G., AND E. T. GARSIDE. 1975. Lethal levels of pH for brook trout *Salvelinus fontinalis*, Mitchill. Can. J. Zool. 53: 639–641.

DONALDSON, E. M., U. H. M. FAGERLUND, AND J. R. MCBRIDE. 1984. Aspects of the endocrine stress response to pollutants in salmonids. *In* V. W. Cairns et al. [ed.] Contaminants effects on fisheries. Vol. 16. Advances in environmental science and technology. Wiley-Interscience Publications, New York, NY. 213 p.

EVANS, R. E., S. B. BROWN, AND T. J. HARA. 1988. The effects of aluminum and acid on the gill morphology in rainbow trout, *Salmo gairdneri*. Environ. Biol. Fishes. 22: 299–311.

FRENETTE, J., AND J. J. DOBSON. 1984. Brook trout (*Salvelinus fontinalis*) population structure in acidified lac Tantaré, Quebec. Can. J. Fish. Aquat. Sci. 41: 865–877.

HEATH, A. G. 1987. Water pollution and fish physiology. Chap. 1. CRC Press, Inc., Boca Raton, FL.

HIRANO, T., P. PRUNET, H. KAWAUCHI, A. TAKAHASHI, T. OGASAWARA, J. KUBOTA, R. S. NISHIOKA, H. A. BERN, K. TAKADA, AND S. ISHII. 1985. Development and validation of salmon prolactin radioimmunoassay. Gen. Comp. Endocrinol. 59: 266–276.

HONTELA, A., AND K. LEDERIS. 1985. Diel variations in arginine vasotocin content of goldfish brain and pituitary: effects of photoperiod and pinealectomy. Gen. Comp. Endocrinol. 57: 397–404.

HONTELA, A., Y. ROY, R. VAN COILLIE, K. LEDERIS, AND G. CHEVALIER. 1989. Differential effects of low pH and aluminium on the caudal neurosecretory system of the brook trout, *Salvelinus fontinalis*. J. Fish Biol. 35: 265–273.

KELSO, J. R. M., C. K. MINNS, J. E. GRAY, AND M. L. JONES. 1986. Acidification of surface waters in Eastern Canada and its relationship to aquatic biota. Can. Spec. Publ. Fish. Aquat. Sci. 87: 42 p.

LAHLOU, B., AND A. GIORDAN. 1970. Le contrôle hormonal des échanges et de la balance de l'eau chez le Teleostéen d'eau douce *Carassius auratus*, intact et hypophysectomisé. Gen. Comp. Endocrinol. 14: 491–509.

LEDERIS, K. 1984. The fish urotensins: hypophyseal and peripheral actions in fishes and mammals, p. 247–263. *In* L. Martini and W. F. Ganong [ed.] Frontiers in neuroendocrinology. Vol. 8. Raven, New York, NY.

MAETZ, J., J. BOURGUET, B. LAHLOU, AND J. HOUDRY. 1964. Peptides neurohypophysaires et osmoregulation chez *Carassius auratus*. Gen. Comp. Endocrinol. 4: 508–522.

MALTE, H. 1986. Effects of aluminium in hard, acid water on metabolic rate, blood gas tensions and ionic status in the rainbow trout. J. Fish Biol. 29: 187–198.

MCDONALD, D. G., AND C. L. MILLIGAN. 1988. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fish. Aquat. Sci. 45: 1606–1613.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Toxic efects of aluminium on the brown trout, *Salmo trutta* L., p. 320–321. *In* Proc. Int. Conf. Ecol. Impact Acid Precip., Norway, SNSF Project, Oslo.

NEVILLE, C. M., AND P. G. C. CAMPBELL. 1988. Possible mechanisms of aluminum toxicity in a dilute acidic environment to fingerlings and older life stages of salmonids. Water Air Soil Pollut. 42: 311–327.

OLIVEREAU, M. 1967. Réactions observées chez l'Anguille maintenue dans un milieu privé d'électrolytes, en particulier au niveau du système hypothalamo–hypophysaire. Z. Zellforsh. 80: 264–285.

OLIVEREAU, M., J. M. OLIVEREAU, AND C. AIMAR. 1982. Influence of deionized water supplemented or not with different ions on prolactin cell activity and osmotic regulation in the goldfish. Comp. Biochem Physiol. 71A(1): 11–16.

RODGERS, D. W. 1984. Ambient pH and calcium concentrations as modifiers of growth and calcium dynamics of brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 41: 1774–1780.

SADLER, K., AND S. LYNAM. 1987. Some effects on the growth of brown trout from exposure to aluminium at different pH levels. J. Fish Biol. 31: 209–219.

SAWYER, W. H. 1977. Evolution of neurohypophyseal hormones and their receptors. Fed. Proc. 36: 1842–1847.

SAWYER, W. H., M. UCHIYAMA, AND P. K. T. PANG. 1982. Control of renal functions in lungfishes. Fed. Proc. 41: 2361–2364.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminium toxicity to brook trout (Salvelinus fontinalis) in acidified waters, p. 341–366. *In* T. Y. Toribara et al. [ed.] Polluted rain. Plenum, New York, NY.

SZABO, Z., AND B. MOLNAR. 1965. Experimental investigations on neurosecretion in mudfish (*Misgurnus fossilis* L.). Acta Biol. 15: 383–392.

WALKER, R. L., C. M. WOOD, AND H. L. BERGMAN. 1988. Effects of low pH and aluminum on ventilation in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1614–1622.

WENDELAAR BONGA, S. E., P. H. M. BALM, AND G. FLIK. 1988. Control of prolactin secretion in the teleost *Oreochromis mossambicus*: effects of water acidification. Gen. Comp. Endocrinol. 72: 1–12.

WENDELAAR BONGA, S. E., C. J. M. LOWIK, AND J. C. A. VAN DER MEIJ. 1983. Effects of external Mg^{++} and Ca^{++} on branchial osmotic water permeability and prolactin secretion in the teleost fish *Sarotherodon mossambicus*. Gen. Comp. Endocrinol. 52: 222–231.

WOOD, C. M. 1987. The physiological problems of fish in acid waters. *In* R. Morris, D. J. A. Brown, E. W. Taylor, and J. A. Brown [ed.] Acid toxicity and aquatic animals. Society for experimental biology seminar series. Cambridge University Press, London.

WOOD, C. M., D. G. MCDONALD, C. E. BOOTH, B. P. SIMONS, C. G. INGERSOLL, AND H. L. BERGMAN. 1988. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587–1596.

Interbreeding with Domestic Strain Increases Foraging under Threat of Predation in Juvenile Steelhead Trout (*Oncorhynchus mykiss*): An Experimental Study

Jörgen I. Johnsson[1] and Mark V. Abrahams[2]

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Johnsson, J. I., and M. V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Can. J. Fish. Aquat. Sci. 48: 243–247.

The foraging behaviour of laboratory-reared juvenile steelhead trout (*Oncorhynchus mykiss*) and steelhead/domesticated rainbow trout hybrids were compared. In 10 replicate experiments, 10 fish from each strain were allowed to choose between foraging in a safe area or an area containing a predator. The hybrid trout were significantly more willing to risk exposure to the predator than were the steelhead. It was possible that differences in the relative willingness to risk exposure may have reflected differences in their susceptibility to predation. A second experiment measured the susceptibility of these two strains to the predator by simulating standardized encounters between predator and prey. Both strains suffered identical mortality rates and therefore were considered to be equally susceptible to the predator. This experiment confirmed that the hybrid trout were significantly more willing to take risks than the wild steelhead. These results indicate that interbreeding between escaped hatchery and wild fish may have a potentially damaging effect on the wild population.

On a comparé le comportement de recherche de nourriture de la truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) et d'hybrides de cette truite avec la truite arc-en-ciel d'élevage. Dans le cadre de 10 sous-expériences, 10 poissons de chaque souche ont pu choisir entre la recherche de nourriture dans une zone sécuritaire ou une zone abritant un prédateur. Les truites hybrides étaient nettement plus portées à s'exposer au prédateur que les truites arc-en-ciel anadromes. Il se peut que les écarts d'exposition traduisent des différences de prédisposition à la prédation. On a tenté au moyen d'une deuxième expérience de quantifier la prédisposition de ces deux souches à la prédation en simulant des rencontres normalisées entre prédateur et proie. Comme les deux souches ont fait l'objet de taux identiques de mortalité, on les a considérées également sujettes à la prédation. Cette expérience a confirmé l'hypothèse selon laquelle les truites hybrides sont nettement plus prédisposées à prendre des risques que les truites arc-en-ciel anadromes sauvages. Les résultats révèlent que le croisement de poissons d'élevage en liberté et de poissons sauvages pourrait nuire aux populations sauvages.

Received March 8, 1990
Accepted August 20, 1990
(JA499)

Reçu le 8 mars 1990
Accepté le 20 août 1990

Recent research in behavioural ecology has demonstrated that foraging behaviour is significantly altered by risk of predation (see Lima and Dill 1990 for a recent review). Responses to predator intimidation include alteration of the prey's diet (Dill and Fraser 1984), of foraging mode (Valone and Lima 1987), and of the time (Lima 1988) and location of foraging (Kotler 1984; Abrahams and Dill 1989). Furthermore, the behavioural modifications generated by predator intimidation can amplify inter- and intraspecific competition. Indeed, risk of predation is now considered to be a major ecological force.

A concern of salmon biologists is that selection in hatcheries may be against phenotypes which are adaptive in the wild, ultimately resulting in reduced returns of hatchery fish. Some evolutionary responses to hatchery environments have already been detected. For example, domesticated brook trout (*Salvelinus fontinalis*) are less sensitive to human-caused disturbance, are more oriented to the water surface (Vincent 1960; Moyle 1969), and are also more aggressive than wild trout (Moyle 1969). Swain and Riddell (1990) have also observed increased aggressiveness in hatchery-reared coho salmon (*Oncorhynchus kisutch*).

Here, we compare the foraging behaviour of a wild strain and a hybrid wild/domesticated strain of juvenile rainbow trout (*Oncorhynchus mykiss*). In freshwater, juvenile trout are territorial, opportunistic feeders (Hartman 1965; Antonelli et al. 1972) that are exposed to a variety of predators. In the wild, these trout must continuously make trade-offs between feeding and risking exposure to predators. However, in a hatchery, fish are raised in an environment containing no predators and must compete for food thrown on the water surface. Cautious fish tend to remain lower in the water column (Vincent 1960) where they are less able to compete for food. Selection for high growth rate by hatcheries (Gall and Huang 1988; Johnsson and Clarke 1988; Gjerde and Schaeffer 1989) may therefore select against predator avoidance. Consequently, our hypothesis is that domestication increases foraging under threat of predation in trout.

[1]Permanent address: Department of Zoology, University of Göteborg, Box 250 59, S-400 31 Göteborg, Sweden.

[2]Present address: Department of Zoology, University of Manitoba, Winnipeg, Man. R3T 2N2, Canada.

We tested this hypothesis by comparing the relative willingness of wild and hybrid wild/domesticated trout to risk exposure to a predator in order to gain access to additional food. To determine whether the fish from the two strains were taking equivalent risk when exposed to the predator, their susceptibility to the predator in this apparatus was also compared.

Methods

Experimental Strains

Wild steelhead trout were obtained from the winter run population in the Big Qualicum river (1 male, 7 females) and the summer run population in the Puntledge river (10 males, 4 females). Both rivers are situated on the east coast of Vancouver Island, approximately 50 km apart. Eleven rainbow trout males were obtained at HyWave Sea Farm in Prince Rupert from a stock that had been domesticated for more than 20 yr. Half of the eggs from each wild steelhead female were fertilized by a wild male and the other half by a domesticated male. Each male was used only once. Of the resulting 22 half-sib families, 11 were pooled within each male parent type creating the two strains. The proportional contribution of offspring from each female was similar between the strains.

The strains were held in separate 200-L freshwater flowtanks maintained at ambient temperature, decreasing from 17°C (25 August) to 12°C at the end of the experiments (24 October). The fish were fed commercial dry crumbles (White Crest) at a rate of 2–2.5% body weight per day and received a simulated natural photoperiod.

Experiment 1: Foraging and Predation Risk

The influence of predation risk on patch choice was studied in 10 replicate experiments using a procedure developed by M. V. Abrahams and M. C. Healy (unpubl. data). Steelhead ($n = 100$) and hybrids ($n = 100$) of similar fork length (7.1 to 8.7 cm depending on the replicate) were used for this purpose (Table 1). Prior to each experiment, 10 fish from each strain were allowed 3 to 6 d to acclimate to the experimental food, Murex Medium freeze-dried plankton (*Euphausia pacifica*), which was provided once per day at maintenance rations. One day before the experiment, the acclimated fish were anaesthetized in 2-phenoxyethanol and one strain received a freeze brand on the dorsal surface between the head and dorsal fin. Weight and fork length were measured for each individual and the fish were allowed 1 d to recover from the treatment in a 200-L tank. No food was provided during the recovery.

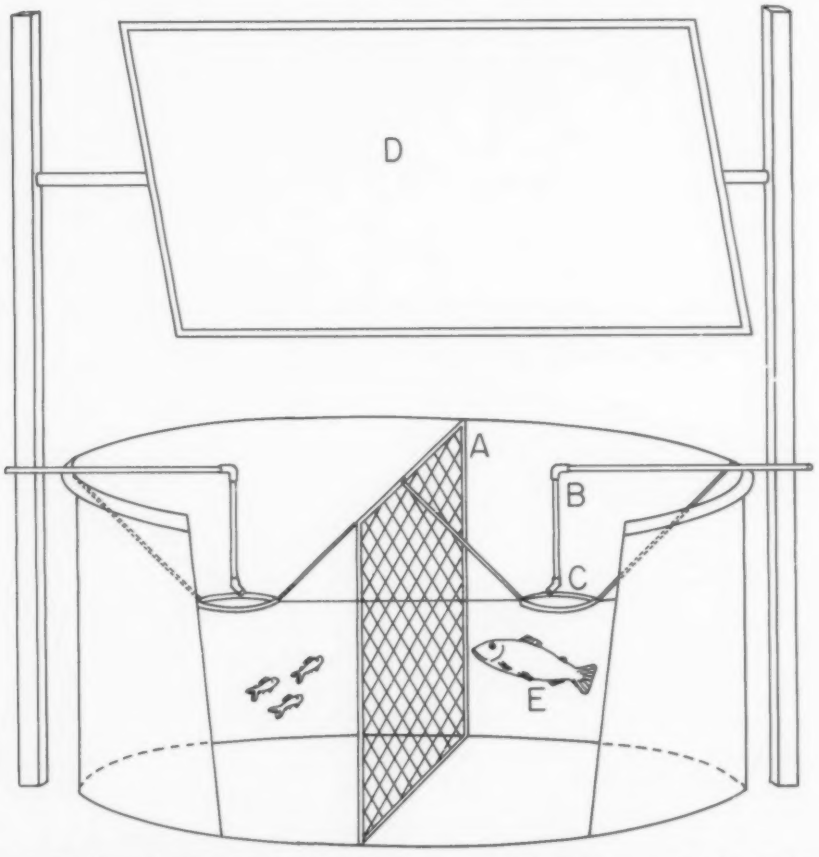


FIG. 1. Experimental tank in the foraging risk experiment. A, dividing net screen; B, tube containing food which could be blown into floating rings (C) on each side using compressed air; D, mirror through which the fishes were observed; E, predator.

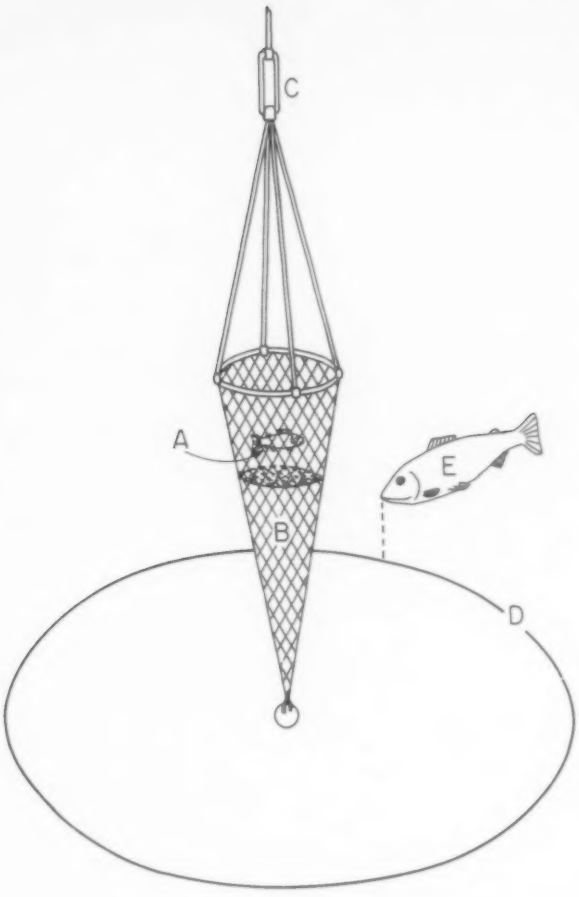


FIG. 2. Predation susceptibility experiment. The juvenile (A) was enclosed in a net (B) which instantly sank to the bottom when the line (C) was released. D, painted circled at bottom of tank to standardize the attack distance of the predator (E).

The following morning the hybrid and steelhead trout were transferred simultaneously to a 2500-L circular experimental tank (Fig. 1). The tank was divided in two by a net screen (mesh size 2.1 cm), through which the juveniles could pass freely, while the predator (an adult rainbow trout) was constrained to one side of the tank. The juveniles were allowed 30 min to investigate the tank before food was presented. Equal amounts of food (0.4 g) were added remotely to both sides of the tank by forcing compressed air through feeding tubes (Fig. 1). No observations were made on the first day to allow the fish to acclimate to the apparatus.

Food was provided at 0900 on the second day. A mirror placed over the tank (Fig. 1) allowed us to record the positions of the fish at 30-s intervals for 25 min. The freeze brand allowed the two strains to be distinguished in the apparatus. These recordings were repeated in four trials per day, separated by 2 h. However, food was only provided in the first trial. After the final trial, all prey fish were removed from the apparatus. Mean frequencies were calculated within each strain and trial by dividing the total number of juveniles recorded in the risky

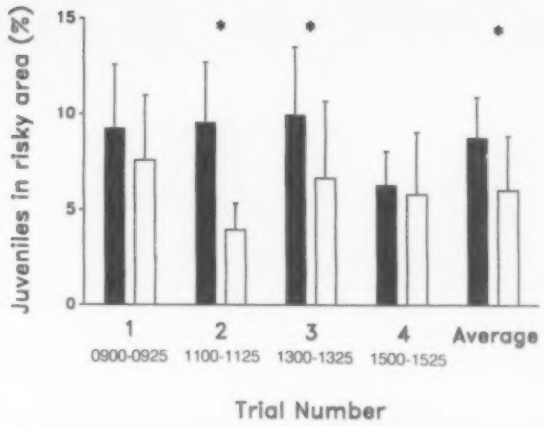


FIG. 3. Mean percent steelhead (open bars) and hybrid trout (solid bars) juveniles present in the risky area (with predator) at four trials in 10 replicate food patch choice experiments. Food was provided at the start of trial 1. Error bars denote standard errors of mean frequencies. Differences among strains were tested using the one-tailed Wilcoxon matched-pairs signed-ranks test ($P < 0.05$).

area by the number of observations (= 50). Ten replicate experiments, each with 10 fish from each strain, were conducted between 13 September and 1 October 1989.

Experiment 2: Susceptibility to Predation

To determine whether the steelhead and hybrid trout were equally susceptible to the predator, we exposed both strains to a standardized encounter with the predator and estimated their probability of escape. Experiments were conducted in a 2500-L circular tank, 2 m in diameter, which contained a predator (an 850-g rainbow trout).

Figure 2 illustrates the conical enclosure developed by M. V. Abrahams and M. C. Healey (unpubl. data) to constrain the prey in the centre of the tank. A circle was drawn on the bottom of the tank, 46 cm from the enclosure. When the predator placed its nose on the line, the prey was released. If the prey survived for 10 s, it was considered to have escaped.

Experiments were conducted between 10 and 24 October 1989. Six juveniles were tested each day. One day prior to each experiment, similar-sized hybrid and steelhead trout were removed from the holding tanks and anaesthetized in 2-phenoxyethanol to obtain their weight and fork length. After handling, the two strains were kept in separate 200-L tanks without being fed. Experiments were conducted between 1300 and 1400 the next day. Steelhead and hybrid trout were presented in alternating order to the predator and the relative order was alternated each day to minimize effects of variations in predatory motivation.

Results

Experiment 1: Foraging and Predation Risk

During these experiments, three steelhead trout and one hybrid were killed by the predator. On average over all trials, the hybrids were more frequently observed on the risky side (one-tailed Wilcoxon matched-pairs signed-ranks test, $n = 10$,

$P<0.05$). Within trials, the hybrids were significantly more risk prone during trials 2 and 3 (one-tailed Wilcoxon matched-pairs signed rank test, $P<0.04$ for both trials), but not during trials 1 and 4 ($P>0.1$ for both trials, Fig. 3). On average, 9% of the hybrids occupied the risky side compared with 6% of the juvenile steelheads. No significant effect of trials on the frequency of juveniles in the risky area was found when all four observation periods were compared for each strain separately (Friedman two-way analysis of variance by ranks, $n=10$, $P>0.3$ for both strains). Size was not significantly different among strains in any replicate (Table 1, Mann-Whitney U-test, $P>0.1$ for all groups). Freeze branding had no effect on the relative willingness of individuals within a strain to risk exposure to the predator (Mann-Whitney U-test, $P>0.2$ for all tests).

Experiment 2: Susceptibility to Predation

The mean weight of the hybrid and steelhead juveniles used in the trials was 12.4 g (SE 0.4) and 12.3 g (SE 0.4), respectively. Of 45 individuals tested from each strain, 29 hybrids and 29 steelheads (or 64%) escaped. Thus, no difference existed in susceptibility to the predator between the strains.

Discussion

This study demonstrated that a hybrid wild/domesticated strain of trout was significantly more willing to risk exposure to a predator than a wild strain of trout. Furthermore, since both the wild trout and the hybrid trout were reared under identical conditions and were half-sib progeny from wild steelhead females, the behavioural difference is inherited from the male parental strain. We tested an alternative hypothesis that the hybrid trout were less vulnerable to the predator and thus inhabited the risky area with a lower cost than the steelhead. This hypothesis was rejected, as both strains were equally vulnerable to predator attacks. Increased risk proneness without a simultaneous improvement in predator avoidance ability is likely to reduce the survival of hybrid fish in natural habitats. Size differences can influence predation risk and behavioural responses in fish (Werner et al. 1983; Hargreaves and LeBrasseur 1986) but were minimized in this study, since both strains were the same size.

Since the first encounter with a predator may be lethal, it is essential that the young animal behaves appropriately without experience of the situation (Slater 1983). This view is supported by the high survival of inexperienced juveniles in our predation susceptibility experiment and the observation that virtually all juveniles in the foraging experiments occupied the safe area in the tank after 30 min of exposure to the predator. However, predator avoidance in salmon is enhanced by learning. Olla and Davis (1989) demonstrated that hatchery-reared coho salmon avoid predation from lingcod (*Ophiodon elongatus*) better after conditioning to live predators and predation-associated stimuli. In our foraging experiments the juveniles were exposed to the experimental conditions for 24 h before observations were made. We do not know how behavioural differences caused by domestication are influenced by the time of exposure to predators, an important consideration to predict the fitness of hybrid salmonids in natural habitats.

It is possible that the observed differences among the strains were caused by life history differences unrelated to domestication. Interpopulation differences in aggressive behaviour have been described for coho salmon (Rosenau and McPhail 1987; Swain and Holtby 1989). However, our results are consistent with those of Swain and Riddell (1990) who observed higher aggression in hatchery stocks compared with wild coho salmon. Both aggressive behaviour and foraging in the presence of a predator increase susceptibility to predation under natural conditions, but are not disadvantageous in domesticated environments and may even be selected for indirectly if they enhance growth through improved competitive ability (Moyle 1969; Kinghorn 1983). Hence, the direction of behavioural differences found between wild and hatchery populations is in agreement with adaptive arguments.

Our results are of interest in light of the increasing numbers of escaped farm salmon, which may affect the gene pools of natural salmon populations if interbreeding occurs. Increased willingness to risk exposure to predators will ultimately reduce survival of the offspring from such crosses, which would decrease natural production. Possible effects of behavioural changes should also be considered when hatchery juveniles are used to rebuild natural populations (see Nickelson et al. 1986). Reisenbichler and McIntyre (1977) found lower survival in a stream by the offspring of hatchery-reared steelhead trout and hatchery/wild trout hybrids than by wild offspring after only

TABLE 1. Mean weight (SE), fork length (SE), and average frequency in risky area (with predator) in juvenile steelhead trout (S) and steelhead/domesticated rainbow trout hybrids (H) in 10 food patch choice experiments. Ten fish from each strain were pooled in each replicate. The identity of the freeze-branded strain is indicated for each experiment.

Replicate No.	Weight (g)		Length (cm)		Branded strain	Frequency (%) in risky area	
	H	S	H	S		H	S
1	4.4 (0.3)	4.3 (0.2)	7.1 (0.2)	7.3 (0.1)	H	10.9	3.6
2	4.5 (0.2)	4.5 (0.2)	7.2 (0.1)	7.4 (0.1)	S	22.6	12.2
3	4.5 (0.3)	4.5 (0.4)	7.1 (0.1)	7.3 (0.2)	H	2.5	2.4
4	4.9 (0.3)	4.8 (0.5)	7.3 (0.2)	7.4 (0.2)	S	1.5	1.0
5	6.6 (0.4)	6.6 (0.4)	7.9 (0.1)	8.1 (0.2)	H	7.2	1.8
6	5.9 (0.5)	5.7 (0.4)	7.7 (0.2)	7.8 (0.2)	S	1.2	0
7	6.8 (0.5)	7.3 (0.6)	8.1 (0.2)	8.4 (0.2)	H	16.8	29.8
8	6.2 (0.5)	6.1 (0.4)	7.9 (0.2)	8.0 (0.2)	S	10.4	4.5
9	8.0 (0.7)	7.9 (0.6)	8.6 (0.2)	8.7 (0.2)	H	6.5	2.4
10	7.4 (0.5)	7.6 (0.4)	8.3 (0.2)	8.6 (0.1)	S	8.1	2.6

two generations of hatchery rearing. Further, in a study by Chilcote et al. (1986), the success of hatchery steelhead in producing smolt offspring was only 28% of that for wild fish. Future studies should examine the extent of behaviour changes caused by domestication and how such changes affect production in natural and artificial aquatic systems.

Acknowledgements

Eggs and milt for the experimental crosses were collected and transported by Bill Damon, John Morgan, Harry Orenius, and Alvin Solmie. Craig Clarke and Brian Riddell gave helpful comments on the manuscript. Jörgen Johnsson was supported by grants from the Swedish Institute "Oscar Ekmans Understödsfond," "Helge Ax:son Johnsons Stiftelse," and the Swedish Council for Forestry and Agricultural Research. Mark Abrahams was supported by a Visiting Fellowship to a Canadian Government Laboratory.

References

ABRAHAMS, M. V., AND L. M. DILL. 1989. A determination of the energetic equivalence of the risk of predation. Ecology 70: 999–1007.

ANTONELLI, A. L., R. A. NUSSBAUM, AND S. D. SMITH. 1972. Comparative food habits of four species of streamdwelling vertebrates (*Dicamptodon ensatus*, *D. copel*, *Cottus tenuis*, *Salmo gairdneri*). Northwest. Sci. 46: 277–289.

CHILCOTE, M. W., S. A. LEIDER, AND J. J. LOCH. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans. Am. Fish. Soc. 115: 726–735.

DILL, L. M., AND A. H. G. FRASER. 1984. Risk of predation and the feeding behaviour of juvenile coho salmon (*Oncorhynchus kisutch*). Behav. Ecol. Sociobiol. 16: 65–71.

GALL, G. A. E., AND N. HUANG. 1988. Heritability and selection schemes for rainbow trout: body weight. Aquaculture 73: 43–56.

GJERDE, B., AND L. R. SCHAEFFER. 1989. Body traits in rainbow trout. II. Estimates of heritabilities and of phenotypic and genetic correlations. Aquaculture 80: 25–44.

HARGREAVES, N. B., AND R. J. LEBRASSEUR. 1986. Size selectivity of coho (*Oncorhynchus kisutch*) preying on juvenile chum (*O. keta*) salmon. Can. J. Fish. Aquat. Sci. 43: 581–586.

HARTMAN, G. F. 1965. The role of behavior in the ecology and interaction of underyearling coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 22: 1035–1081.

JOHNSSON, J., AND W. C. CLARKE. 1988. Development of seawater adaptation in juvenile steelhead trout (*Salmo gairdneri*) and domesticated rainbow trout (*Salmo gairdneri*) — effects of size, temperature and photoperiod. Aquaculture 71: 247–263.

KINGHORN, B. P. 1983. A review of quantitative genetics in fish breeding. Aquaculture 31: 283–304.

KOTLER, B. P. 1984. Risk of predation and the structure of desert rodent communities. Ecology 65: 689–701.

LIMA, S. L. 1988. Vigilance and diet selection: a simple example in the dark-eyed junco. Can. J. Zool. 66: 593–596.

LIMA, S. L., AND L. M. DILL. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68: 619–640.

MOYLE, P. B. 1969. Comparative behavior of young brook trout of domestic and wild origin. Prog. Fish-Cult. 31: 51–59.

NICKELSON, T. E., M. F. SOLAZZI, AND S. L. JOHNSON. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in oregon coastal streams. Can. J. Fish. Aquat. Sci. 43: 2443–2449.

OLLA, B. L., AND M. W. DAVIS. 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. Aquaculture 76: 209–214.

REISENBICHLER, R. R., AND J. D. MCINTYRE. 1977. Genetic difference in growth and survival of juvenile hatchery and wild steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 123–128.

ROSENAU, M. L., AND J. D. MCPHAIL. 1987. Inherited differences in agonistic behavior between two populations of coho salmon. Trans. Am. Fish. Soc. 116: 646–654.

SLATER, P. J. B. 1983. The development of individual behaviour, p. 82–113. *In* T. R. Halliday and P. J. B. Slater [ed.] Animal behaviour. Vol. 3 Genes, development and learning. W. H. Freeman and Company, New York and San Francisco.

SWAIN, D. P., AND L. B. HOLTBY. 1989. Differences in morphology and behavior between juvenile coho salmon (*Oncorhynchus kisutch*) rearing in a lake or in its tributary stream. Can. J. Fish. Aquat. Sci. 46: 1406–1414.

SWAIN, D. P., AND B. E. RIDDELL. 1990. Variation in agonistic behaviour between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 47: 566–571.

VALONE, T. J., AND S. L. LIMA. 1987. Carrying food items to cover for consumption: the behaviour of ten bird species feeding under the risk of predation. Oecologia 71: 286–294.

VINCENT, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Trans. Am. Fish. Soc. 89: 35–52.

WERNER, E. E., J. F. GILLIAM, D. J. HALL, AND G. G. MITTELBACH. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540–1548.

Survival, Contribution, and Return of Hatchery Coho Salmon (*Oncorhynchus kisutch*) Released into Freshwater, Estuarine, and Marine Environments

Mario F. Solazzi, Thomas E. Nickelson, and Steven L. Johnson

Research and Development Section, Oregon Department of Fish and Wildlife, 850 S.W. 15th Street, Corvallis, OR 97333, USA

Solazzi, M. F., T. E. Nickelson, and S. L. Johnson. 1991. Survival, contribution, and return of hatchery coho salmon (*Oncorhynchus kisutch*) released into freshwater, estuarine, and marine environments. Can. J. Fish. Aquat. Sci. 48: 248–253.

We released six groups of marked yearling hatchery coho salmon (*Oncorhynchus kisutch*) in six locations each year for five years beginning with the 1981 brood. Fish were released immediately below Bonneville Dam (control), at the Tongue Point Coast Guard Station (head of saltwater intrusion in the Columbia River), between the jetties at the Columbia River bar, in the Columbia River plume water, in coastal water approximately 19 km north and 19 km offshore of the mouth of the river, and in oceanic water approximately 38 km offshore. We found a 1.6-fold increase in the survival index (ocean catch through September 18 each year) for the fish released at Tongue Point compared with the control group. After adjusting for differences in the survival index between release groups, we found a 2.5-fold increase in the contribution to the Columbia River gillnet fishery from the fish released at Tongue Point compared with the control group. We found no significant difference between survival of the other release groups and survival of the control group. We also found that the percentage of adult fish that returned to locations other than the Columbia basin increased as the distance the fish were transported offshore increased.

Pendant cinq années consécutives à partir de 1981, six groupes de saumon coho (*Oncorhynchus kisutch*) d'un an produits en pisciculture ont été relâchés, après marquage, à six endroits. Le lâcher a été effectué immédiatement en aval du barrage de crue de Bonneville, au poste de garde-côte de Tongue Point (au sommet du coin d'eau salée du fleuve Columbia), entre les brises-lames à la barre du fleuve Columbia, dans le panache du fleuve Columbia, dans les eaux côtières à environ 19 km au nord et au large de l'embouchure du fleuve et dans les eaux océaniques à environ 38 km au large. On a noté une augmentation de 1,6 fois de l'indice de survie (prises effectuées en mer le 18 septembre de chaque année) chez les poissons relâchés à Tongue Point par rapport au groupe témoin. Après correction des résultats en fonction des différences de l'indice de survie chez les différents groupes, on a noté, par rapport au groupe-témoin, une augmentation de 2,5 fois des prises au filet maillant dans le fleuve Columbia attribuable aux poissons lâchés à Tongue Point. Aucune différence significative des taux de survie n'a été décelée entre les autres groupes expérimentaux et le groupe témoin. On a aussi noté que le pourcentage de poissons adultes revenant à des endroits autres que le bassin du Columbia avait augmenté en fonction de l'éloignement du point de lâcher en haute mer.

Received March 26, 1990
Accepted August 20, 1990
(JA524)

Reçu le 26 mars 1990
Accepté le 20 août 1990

Survival rates of yearling coho salmon (*Oncorhynchus kisutch*) released from Columbia River hatcheries have historically fluctuated from a high of over 10% to a low of less than 1% (Johnson 1982). Changes in ocean conditions, especially upwelling, have been identified as a major factor determining survival of juvenile salmon (Nickelson 1986; Fisher and Pearcy 1988). However, the specific mechanism(s) by which ocean conditions influence survival are still poorly understood.

In Sweden and Norway, releases of hatchery-reared Atlantic salmon (*Salmo salar*) smolts directly into estuarine and marine waters have resulted in increased survival when compared with similar releases made in fresh water (Eriksson et al. 1981; Hansen 1982; Gunnerod et al. 1988). Macdonald et al. (1988) and Levings et al. (1989) presented preliminary information from river, estuarine, and marine releases of chinook salmon (*Oncorhynchus tshawytscha*) near Campbell River, British Columbia, Canada. They speculated that releases into the estuary may have a positive effect on survival. This effect is thought to be related to decreased predation and stress, increased food availability, and ease of osmoregulation in the estuary. However, the effect on survival to adult is still unknown.

We designed an experiment to identify the general location (river, estuary, or ocean) where a survival "bottleneck" might occur for hatchery-reared coho salmon in the Columbia River system. In addition, we wanted to determine if total contribution to the in-river fisheries was different among the release groups. We chose release sites based on differences in oceanographic features of the major water types that are associated with the Columbia River and its discharge into the Pacific Ocean.

As fresh water from the Columbia River enters the ocean during the early summer months, a plume of low-salinity water protrudes out to sea and to the south along the Oregon coast. Near the mouth of the Columbia River, this water tends to be rich in suspended particles and is often a muddy-green or green

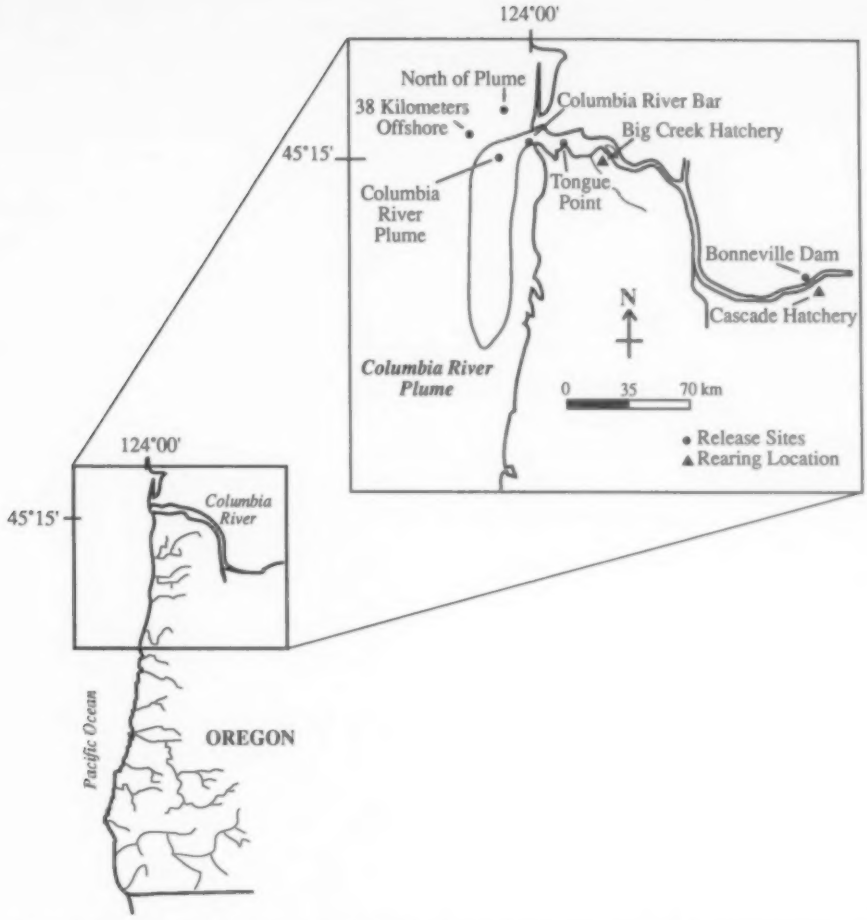


FIG. 1. Release sites and rearing locations for the juvenile coho salmon released during the ocean release experiment.

color. The plume water generally produces an obvious color and thermal front where it meets offshore waters or deep blue oceanic waters. The nearshore waters (north of the Columbia River plume influence) tend to resemble coastal upwelled waters and have different salinity and temperature profiles than do the other water types described. Pearcy and Mueller (1970) presented a more detailed description of the Columbia River plume, nearshore, and oceanic waters off the Oregon coast during the summer.

Methods

We released six groups of marked (coded-wire tagged and adipose fin clipped) coho salmon each year for five consecutive years in six locations beginning with the 1981 brood released in 1983 (Fig. 1). The release sites were immediately below Bonneville Dam at river kilometre (RK) 234 (control), at the Tongue Point Coast Guard Station (RK 29, head of saltwater intrusion), between the jetties at the Columbia River bar (RK 2), in the Columbia River plume water, in coastal water approximately 19 km north and 19 km offshore of the mouth of the river (north of the plume), and in oceanic water approximately 38 km offshore.

The 1981–83 broods were reared and tagged at Cascade Hatchery and the four groups to be released offshore were trucked from Cascade Hatchery to Big Creek Hatchery 2–4 wk prior to release. The 1984 and 1985 broods were reared and tagged at Big Creek Hatchery. All groups were reared in concrete raceways at an approximate density of 687 fish/m^3. Between October and November, fish from each release group were marked with adipose fin clips and different coded-wire tags.

Saltwater challenge experiments (Clark and Blackburn 1977; Blackburn and Clark 1987) demonstrated that yearling coho salmon smolts (of sufficient size) were able to survive in full-strength seawater by 1 June. We therefore released all fish between 12 May and 7 June. The average size of fish at release ranged from 24 to 38 g (Table 1). The difference in average sizes among the 1981 brood was due to additional growth the offshore groups obtained while in the warmer water at Big Creek Hatchery. Adjustments in feeding schedules and rations for each group reduced this size difference in subsequent years. A staggered release schedule was used so that the up-river release groups (below Bonneville Dam and at Tongue Point) would reach the ocean at approximately the same time that the other groups were released. The control group was always

TABLE 1. Release statistics for groups of yearling hatchery coho salmon released in the Columbia River and Pacific Ocean, 1983–87.

Release year	Treatment group	Date released	Number released	Size at release (g)
1983	Control	23 May 1983	43 126	25.0
	Tongue Point	2 June 1983	43 167	24.0
	Bar	6 June 1983	42 575	35.0
	Plume	8 June 1983	42 230	34.0
	North of plume	6 June 1983	42 185	33.0
	38 km	7 June 1983	43 215	33.0
1984	Control	18 May 1984	42 765	35.5
	Tongue Point	25 May 1984	42 517	35.7
	Bar	31 May 1984	43 232	37.5
	Plume	31 May 1984	42 913	37.8
	North of plume	30 May 1984	42 926	36.6
	38 km	29 May 1984	42 015	36.3
1985	Control	17 May 1985	39 760	31.7
	Tongue Point	24 May 1985	39 850	31.5
	Bar	31 May 1985	39 328	33.3
	Plume	31 May 1985	39 709	32.6
	North of plume	30 May 1985	39 563	31.9
	38 km	29 May 1985	39 722	31.9
1986	Control	20 May 1986	37 326	35.2
	Tongue Point	29 May 1986	37 328	34.9
	Bar	4 June 1986	39 848	35.5
	Plume	4 June 1986	37 893	36.0
	North of plume	5 June 1986	39 873	36.3
	38 km	3 June 1986	39 878	35.5
1987	Control	12 May 1987	39 553	32.7
	Tongue Point	19 May 1987	39 662	33.4
	Bar	27 May 1987	39 580	34.1
	Plume	27 May 1987	39 518	33.9
	North of plume	28 May 1987	39 556	34.9
	38 km	26 May 1987	39 306	33.6

released 2 wk before the groups released at the Columbia River bar and the three release groups released directly into the ocean. The group released at Tongue Point was released 1 wk before these groups. In 1983, a National Marine Fishery Service (NMFS) sampling crew captured 21 juvenile coho salmon from the control group at RK 74 (Jones Beach) between 29 May and 12 June (Dawley 1983). These data confirmed that the control fish did enter the ocean at about the same time that the other groups were released into the ocean.

On the day of release each group to be transported was crowded to one end of the hatchery raceway and lifted via a fish pump into a transport truck. The control and Tongue Point groups were trucked from the rearing hatchery and released directly into the Columbia River. Average transport time from Cascade hatchery to the control release site was approximately 1 h and to Tongue Point 3–4 h. Transport time from Big Creek Hatchery to the control release site was about 3–4 h and to Tongue Point 0.75 h.

The groups released at the bar and at the three ocean release sites were treated the same as the other two groups except that the fish were transferred from the truck into two 6000-L polyethylene tanks secured by the tow winch and auxiliary cables to the fantail of an oceangoing tugboat. Transport time from Big Creek Hatchery to the boat at Pier 1 in Astoria, Oregon, was about 1 h.

The deck tanks were fitted with water inlet lines capable of replacing the water in the tanks once each hour. As ambient water was pumped into the tanks the overflow was allowed to spill onto the deck. Each tank was also supplied with oxygen through three carbon stones secured to the tank bottom. Oxygen was supplied from cylinders secured to the deck railing. A manifold with valves and pressure gauges for each carbon stone allowed us to equalize the oxygen flow to each tank. A dissolved oxygen probe was suspended in one tank and oxygen level was monitored regularly from the wheelhouse of the tugboat. Dissolved oxygen level was maintained above 10 ppm and averaged approximately 12 ppm. The floors of the deck tanks were sloped toward the drain, and fish were allowed to leave the tank through a large hose routed through the scuppers. The drain hose ended flush with the water surface, and except during rough ocean conditions, the fish were released without splashing into the water. Replacement water pumps remained on until all fish had left the tank (generally within 15–20 min).

Transport time for the deck tank groups varied depending on release location and ocean and weather conditions. Average transport time (from Pier 1) was about 1 h for the bar group, 1.5–2 h for the plume groups, and 3–4 h for the 38 km at sea and north of plume groups.

As the fish were released from the deck tanks we obtained a sample of approximately five fish to verify the tag code. In addition, we recorded water temperature, salinity, Secchi depth, water depth, the number of birds present, and the loran C coordinates at the release site.

Estimates of the numbers of adult coho salmon from each release group that were caught in ocean commercial and recreational fisheries were derived from data collected by samplers at ports along the Pacific Coast. Each year, fishery agencies expand the number of observed tags recovered from these fisheries to reflect sampling effort and report these data to the Pacific Marine Fisheries Commission (PMFC). Tags were also recovered from all adipose-clipped coho salmon that returned to Bonneville, Big Creek, and Cascade hatcheries.

To provide an "index of survival" for each group, we combined the ocean recreational and commercial catch estimates and divided each year's estimate by the number of smolts released that year. We then calculated the average for all five years. We used this "index of survival" instead of the more traditional estimate (based on total catch plus total escapement) for two reasons: (1) fish transported offshore might demonstrate a different timing and magnitude of return when compared with controls and (2) returns to hatcheries of fish released off-station are often lower than returns of fish released at the hatchery (Slatick et al. 1980, 1982; Johnson et al. 1990). Previous studies on return of adult salmon to site of smolt release (Foerster 1936; Donaldson and Allen 1957) led us to expect a lower rate of return to the Columbia River from smolts released offshore than from smolts released in the river. We therefore estimated survival based on ocean catch during the period before the fish normally enter the Columbia River. To insure that all release groups were exposed to the same harvest rate, we used the catch data only through 18 September each year.

We also examined the catch of each group in the lower Columbia River gillnet fishery by dividing the total net catch each year for each group by the number released to estimate the contribution rate to the fishery. To determine if some groups were caught more than others in the gillnet fishery, independent of their survival, we also examined the gillnet data by dividing the gillnet catch by the survival index to account for differences in ocean survival among the release groups.

We used a two-way analysis of variance (ANOVA) with years as replicates (Snedecor and Cochran 1967) to determine

TABLE 2. Average ocean catch, average gillnet catch, and adjusted gillnet catch divided by the number of fish released each year at each release location. Standard deviation is in parentheses.

Fishery	Release location					
	Control	Tongue Point	Bar	Plume	North of plume	38 km
Ocean	0.0096 (0.0057)	0.0150 (0.0109)	0.0096 (0.0082)	0.0110 (0.0080)	0.0120 (0.0129)	0.0067 (0.0072)
Gillnet	0.0076 (0.0089)	0.0250 (0.0276)	0.0095 (0.0123)	0.0091 (0.0110)	0.0110 (0.0164)	0.0058 (0.0092)
Adjusted gillnet	0.8210 (0.5896)	2.0330 (1.5228)	1.0820 (0.6318)	0.7390 (0.3932)	0.6510 (0.4136)	0.6210 (0.3769)

TABLE 3. Number of coho salmon from experimental releases that returned to locations within and outside the Columbia River basin, 1981–83 brood years.

Release location	Recapture site		Percentage outside Columbia River basin
	Columbia River	Outside Columbia River	
Bonneville	1474	1	<0.1
Tongue Point	28	1	3.4
Bar	47	2	4.1
Plume	62	4	6.1
North of plume	79	21	21.0
38 km	30	18	37.5

if significant differences among release sites existed in average survival index, average contribution to net fisheries, and average contribution to net fisheries after adjusting for survival. Use of the ANOVA assumes homogeneity of variances which we confirmed in the survival index and in the net catch data (Bartlett's test, $P > 0.05$) and in the adjusted net catch data after natural log transformation (Barlett's test, $P > 0.05$). We used Fisher's protected least significant difference (FPLSD) test (Peterson 1985) to determine which release group had a survival index or gillnet catch that was significantly different from the control group.

We divided the number of adult fish from each release group for the 1981–83 broods (the 1984–85 brood data were incomplete) that returned to locations outside the Columbia basin by the total number of returning fish to compare straying of each group.

We used a stepwise multiple regression analysis to identify possible relationships between the survival index and environmental conditions at each release site.

Results

We found a significant difference in the survival index among the six release groups (ANOVA, $P = 0.04$). Survival of the Tongue Point release group was significantly higher (FPLSD, $P = 0.03$) than survival of the control group. On the average we observed a 1.6-fold increase in the survival index for the Tongue Point release group compared with the control group (Table 2). The average survival indexes for the plume group and for the north of the plume group were higher than the control group but the differences were not statistically significant (FPLSD, $P = 0.60$ and $P = 0.33$, respectively. Differences in the survival index of groups released at the bar (FPLSD, $P = 0.99$) and at the 38 km at sea site (FPLSD, $P = 0.24$) were similar to or lower than the controls but not statistically significant.

We also found a significant difference among the six treatment groups in the number of fish harvested in the lower river gillnet fishery (ANOVA, $P = 0.01$). We found that the Tongue Point release group was the only group that differed significantly from the control group (FPLSD, $P = 0.01$). Similar results were obtained from the gillnet catch data after adjusting for differences in survival. We found a significant difference (ANOVA, log-transformed numbers, $P = 0.01$) among release groups. The Tongue Point group was again the only group that differed significantly from the control group (FPLSD, log-transformed numbers, $P = 0.01$). On the average, we found a 2.5-fold increase in the contribution of the Tongue Point release group (after adjusting for survival) to the gillnet fisheries when compared with the control group (Table 2). All ANOVA's also revealed statistically significant differences between years among release groups.

Analysis of the adult escapement data suggested that the returns to locations other than the Columbia River increased as the distance the fish were transported in the deck tanks increased (Table 3).

Environmental conditions as represented by temperature, salinity, Secchi depth, water depth, and the number of predatory birds observed varied both among years and among release sites (Table 4). Environmental conditions at a given release site tended to be different from the conditions at any other release site within the same year. We found no significant relationship (multiple regression, $P > 0.05$) between the survival index and environmental conditions at each release site.

Discussion

Our most significant finding was that estuarine releases of coho salmon (Tongue Point), despite increased transportation stress, survived better than did the river releases (control group). These results tend to support the preliminary results of Macdonald et al. (1988) and Levings et al. (1989) that chinook salmon survival could be enhanced by releasing juveniles directly into the estuary. Although the plume group and the north of the plume group survived on the average at a higher rate than the control group, the differences were not large or statistically significant. The group released north of the plume had the highest variability in survival (2.5–0.6 times the control). We observed low survival on the groups released at the Columbia River bar where we observed the largest number of predatory birds (mostly gulls) at the time of release (Table 4). The group released 38 km at sea also had a low average survival when compared with the control group. These fish were released into relatively unproductive oceanic water, deep Secchi depth, and had the longest transportation time in the deck tanks. These two factors combined may have accounted for the decreased survival of this group.

The increased survival of the Tongue Point group compared with the control group and the fact that the groups released in the plume and to the north of the plume tended to survive better

TABLE 4. Average temperature, salinity, Secchi depth, water depth, and number of predatory birds observed at each release location. Standard deviation is in parentheses.

Treatment group	Temperature (°C)	Salinity (‰)	Secchi depth (m)	Water depth (fathoms)	Predatory birds
Tongue Point	14.4 (1.5)	<1 (0)	—	—	31 (67)
Bar	14.1 (1.3)	19.3 (4.5)	6.0 (1.4)	7.8 (1.3)	689 (929)
Plume	13.3 (0.8)	19.7 (4.8)	8.6 (5.4)	36 (5.5)	25 (45)
North of plume	12.8 (0.8)	28.6 (2.3)	18.4 (4.0)	39 (5.5)	13 (22)
38 km	13.4 (1.1)	29.5 (1.5)	20.8 (5.8)	300 (0)	17 (13)

than the control group suggest that a survival bottleneck for hatchery coho salmon probably occurred in the lower Columbia River. This bottleneck may have been caused by predation, possibly by northern squawfish (*Ptychocheilus oregonensis*). Gunnerod et al. (1988) speculated that the increased survival of the Atlantic salmon smolts released in the ocean compared with river and fjord release was likely associated with lower predation on the ocean-released smolts.

However, the survival bottleneck that we have identified within the lower Columbia River does not explain the interannual variation that we observed in survival of our experimental release groups. Survival of the Tongue Point group was always higher than that of the control group, and both varied with ocean conditions. The 1981 brood (1983 release), which was severely affected by the warm ocean temperatures of El Niño, suffered low survival rates for all groups. The 1983 brood (1985 release) experienced more favorable ocean conditions (unusually cool ocean temperature during spring) than any of the other broods, which probably contributed to the high survival observed for all releases that year. The same trend of low survival for the 1981 brood and high survival for the 1983 brood was also observed for coho salmon released from coastal and Columbia River hatcheries for the same brood years (Fisher and Pearcy 1988; Oregon Department of Fish and Wildlife, unpublished data). Although survival of hatchery coho salmon has been linked to ocean conditions (Nickelson 1986), the results of our study do not provide any insight into the mechanisms of this linkage.

Even after adjusting for differences in survival, the Tongue Point releases contributed significantly more returning adult fish to the gillnet fishery than did the other groups (Table 2). The high contribution to the gillnet fishery from the Tongue Point releases suggested that as these fish began their upstream migration they may have milled around in the lower river, hence delaying upstream migration, whereas the control group migrated rapidly through the lower river. A delayed migration would result in these fish (Tongue Point) being exposed to the gillnet fishery for a longer time than the control group and hence they would experience a higher harvest rate. The Tongue Point group produced fewer returns to the hatcheries than did any other group (Table 3).

The four groups released from deck tanks probably experienced a higher level of stress than did the groups released at Tongue Point or at the hatchery. Specker and Schreck (1980) showed that fish exposed to a second stress after transportation had higher levels of cortisol which took longer to return to the baseline than did cortisol in fish exposed to a single episode of stress. In addition to the stress of being transported in trucks, groups released at the bar and offshore received a second stress when they were transferred to the deck tanks from the transportation trucks. Survival of fish released offshore may be improved over river or estuarine releases if a method can be developed to reduce the stress associated with transporting the smolts offshore.

The offshore release groups had the highest number of fish that returned to locations other than the hatchery of rearing (Table 3). In contrast, the control group had the fewest strays and the most fish that returned to the rearing hatchery. Harden Jones (1968) hypothesized that the downstream migrants (smolts) experienced olfactory imprints that were remembered sequentially. Even though the water in the deck tanks was replaced at the rate of once per hour as the fish were transported to the release site, this appeared to be insufficient to allow the returning adults to consistently return to the lower Columbia River or to the rearing hatchery. These observations support the sequential hypothesis for home stream detection. The disruption of the sequence of olfactory stimuli caused by transporting the fish in deck tanks may explain why the fish released from the deck tanks returned to locations other than the lower Columbia River at a higher rate than the fish released in the river. The decrease in percentage returning to the vicinity of the rearing location as the transport distance increased was similar to results presented by Gunnerod et al. (1988) for ocean releases of Atlantic salmon.

Acknowledgements

This study was made possible by the excellent cooperation of Wayne Stedronsky and Dave Reiben and the crews at Cascade and Big Creek hatcheries. We would also like to thank Knappton Corporation and the various skippers and crews of the *Betsy L* and *Cap Evans*. We are also indebted to Jim Martin for his support and assistance with logistics during the first year of releases. The manuscript was improved by helpful suggestions from C. D. Levings, Wayne Burck, and an anonymous reviewer. Thanks also to Mary Buckman for statistical review. This study was funded with Anadromous Fish Funds administered by the National Marine Fisheries Service and the U.S. Fish and Wildlife Service.

References

BLACKBURN, J., AND W. C. CLARK. 1987. Revised procedure for the 24-hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 35 p.

CLARK, W. C., AND J. BLACKBURN. 1977. A seawater challenge test to measure smolting of juvenile salmon. Fish. Mar. Ser. Res. Dev. Tech. Rep. 705: 11 p.

DAWLEY, E. M. 1983. Migrational characteristics and survival of juvenile salmonids entering the Columbia River estuary. Natl. Mar. Fish. Serv. Contract DE-A179-83BP39652, Q. Prog. Rep., July–Sept. 1983, Seattle, WA.

DONALDSON, L. R., AND G. H. ALLEN. 1957. Return of silver salmon, *Oncorhynchus kisutch* (Walbaum) to point of release. Trans. Am. Fish. Soc. 87: 13–22.

ERIKSSON, C., S. HALLGREN, AND S. UPPMAN. 1981. Lekvandring hos odlad lax (*Salmo salar*) utsatt son smolt i Ljusvan och dess mynningsomrade. Skningsinst. Medd. 3: 16 p. (In Swedish, English abstract)

FISHER, J. P., AND W. G. PEARCY. 1988. Growth of juvenile coho salmon (*Oncorhynchus kisutch*) in the ocean off Oregon and Washington, USA, in years of different coastal upwelling. Can. J. Fish. Aquat. Sci. 45: 1036–1044.

FOERSTER, R. E. 1936. The return from the sea of sockeye salmon, *Oncorhynchus nerka*, with special reference to percentage survival, sex proportions and progress of migration. J. Biol. Board Can. 3: 26–42.

GUNNEROD, T. B., N. A. HVIDSTEN, AND T. G. HEGGBERGET. 1988. Open sea releases of Atlantic salmon smolts, *Salmo salar*, in central Norway, 1973–83. Can. J. Fish. Aquat. Sci. 45: 1340–1345.

HANSEN, L. P. 1982. Salmon ranching in Norway, p. 95–108. *In* C. Ericksson, M. P. Ferranti, and P. O. Larsson [ed.] Sea ranching of Atlantic salmon. Commission of the European Communities. COST 46/4 Workshop.

HARDEN JONES, F. R. 1968. Fish migration. Arnold, London. 325 p.

JOHNSON, S. L. 1982. A review and evaluation of release strategies for hatchery reared coho salmon. Or. Dep. Fish. Wildl. Info. Rep. (Fish) 82-5, Portland, OR.

JOHNSON, S. L., M. F. SOLAZZI AND T. E. NICKELSON. 1990. The effects on survival and homing from trucking hatchery yearling coho salmon to release sites. N. Am. J. Fish. Manage. 10(4): 427–433.

LEVINGS, C. D., C. D. MCALLISTER, J. S. MACDONALD, T. J. BROWN, M. S. KOTYK, AND B. A. KASK. 1989. Chinook salmon (*Oncorhynchus tshawytscha*) and estuarine habitat: a transfer experiment can help evaluate estuary dependency, p. 116–122. *In* C. D. Levings, L. B. Holtby, and M. A. Henderson [ed.] Proceedings of the National Workshop on Effects of Habitat Alteration on Salmonid Stocks. Can. Spec. Publ. Fish. Aquat. Sci. 105.

MACDONALD, J. S., C. D. LEVINGS, C. D. MCALLISTER, U. H. M. FAGERLUND, AND J. R. MCBRIDE. 1988. A field experiment to test the importance of estuaries for chinook salmon, *Oncorhynchus tshawytscha*, survival: short-term results. Can. J. Fish. Aquat. Sci. 45: 1366–1377.

NICKELSON, T. E. 1986. Influences of upwelling, ocean temperature, and smolt abundance on marine survival of coho salmon (*Oncorhynchus kisutch*) in the Oregon production area. Can. J. Fish. Aquat. Sci. 43: 527–535.

PEARCY, W. G., AND J. L. MUELLER. 1970. Upwelling, Columbia River plume and albacore tuna, p. 1101–1113. *In* Sixth International Symposium on Remote Sensing of the Environment. University of Michigan, Ann Arbor, MI.

PETERSON, R. G. 1985. Design and analysis of experiments. Marcel Dekker, New York and Basel, 429 p.

SLATICK, E., L. G. GILBREATH, J. R. HARMON, AND K. A. WALCH. 1982. Imprinting salmon and steelhead trout for homing, 1981. Nat. Mar. Fish. Serv. Contract DE-A129-81-BP27891, Annu. Prog. Rep., Seattle, WA.

SLATICK, E., L. G. GILBREATH, AND K. A. WALCH. 1980. Imprinting salmon and steelhead trout for homing, 1979. Natl. Mar. Fish. Serv. Contract DE-A179-80-BP18236, Annu. Prog. Rep., Seattle, WA.

SNEDECOR, G. W. AND W. G. COCHRAN. 1967. Statistical methods. Iowa State University Press, Ames, IA. 593 p.

SPECKER, J. L., AND C. B. SCHRECK. 1980. Stress responses to transportation and fitness for marine survival in coho salmon *Oncorhynchus kisutch* smolts. Can. J. Fish. Aquat. Sci. 37: 765–769.

Evaluation of a Mark–Recapture Method for Estimating Mortality and Migration Rates of Stratified Populations

Robert M. Dorazio and Paul J. Rago

U.S. Fish and Wildlife Service, National Fisheries Research Center, Box 700, Kearneysville, WV 25430, USA

Dorazio, R. M., and P. J. Rago. 1991. Evaluation of a mark–recapture method for estimating mortality and migration rates of stratified populations. Can. J. Fish. Aquat. Sci. 48: 254–260.

We simulated mark–recapture experiments to evaluate a method for estimating fishing mortality and migration rates of populations stratified at release and recovery. When fish released in two or more strata were recovered from different recapture strata in nearly the same proportions, conditional recapture probabilities were estimated outside the [0,1] interval. The maximum likelihood estimates tended to be biased and imprecise when the patterns of recaptures produced extremely "flat" likelihood surfaces. Absence of bias was not guaranteed, however, in experiments where recapture rates could be estimated within the [0,1] interval. Inadequate numbers of tag releases and recoveries also produced biased estimates, although the bias was easily detected by the high sampling variability of the estimates. A stratified tag–recapture experiment with sockeye salmon (*Oncorhynchus nerka*) was used to demonstrate procedures for analyzing data that produce biased estimates of recapture probabilities. An estimator was derived to examine the sensitivity of recapture rate estimates to assumed differences in natural and tagging mortality, tag loss, and incomplete reporting of tag recoveries.

Nous avons simulé des expériences de marquage–recapture pour évaluer une méthode d'estimation de la mortalité et des taux de migration de poissons de populations stratifiées au moment de la libération et de la recapture. Quand des poissons libérés en deux strates et plus étaient recapturés en plusieurs strates de recapture différentes dans à peu près les mêmes proportions, les probabilités conditionnelles de recapture étaient estimées en dehors de l'intervalle [0,1]. Les estimations du maximum de vraisemblance tendaient à souffrir d'un biais et à être imprécises quand la composition des recaptures donnait des surfaces de vraisemblance extrêmement «plate ». Même dans les expériences où les taux de recapture pouvaient être estimés dans l'intervalle [0,1], l'absence de biais n'était pas garantie. Si le nombre d'individus marqués et le nombre de recaptures étaient insuffisants, les estimations souffraient également d'un biais, mais la grande variabilité au sein des échantillons utilisés pour faire les estimations permettait de repéré facilement ce biais. Une expérience de marquage–recapture effectuée avec une population stratifiée de saumon rouge (*Oncorhynchus nerka*) a été menée pour établir des méthodes d'analyse de données imposant un biais aux estimations des probabilités de recapture. Un estimateur a été établi pour évaluer dans quelle mesure la mortalité supposée due au marquage, la perte d'étiquettes et le signalement incomplet des recaptures peuvent influer sur les estimations des taux de recapture.

Received December 4, 1989
Accepted August 15, 1990
(JA385)

Reçu le 4 décembre 1989
Accepté le 15 août 1990

Mark–recapture experiments are often used to reveal movements of fish among geographic regions or strata. The spatial distribution of recoveries provides a qualitative description of the range and frequency of migrations. However, models that distinguish migration and recapture rates explicitly are required to estimate these rates quantitatively. Application of models that fail to include migration explicitly will produce inaccurate estimates of fishing mortality (i.e. recapture) if the assumed rates of natural mortality and other processes responsible for the disappearance of tags fail to account for the actual rate of movement of tagged fish from the region of release. Under these circumstances, estimates of fishing mortality will be erroneously low.

One solution is to release fish from several geographic regions simultaneously with tags that identify the region of release. Tag recoveries in any individual region will depend on the frequency of migrations to that region from all others and on the intensity of fishing in that region. This interdependency was first recognized by Schaefer (1951), who developed mark–recapture estimators for rates of migration and recapture of temporally or spatially stratified populations. Schaefer used recapture rates to estimate abundances of unmarked fish, but the rates can also be used to assess the magnitude of fishing mortality when tag recoveries occur only through fishing. Darroch (1961) extended Schaefer's work by deriving maximum likelihood estimators for the recapture rates and their variances and covariances. Although Darroch proved that the estimators are nearly unbiased when the number of marked fish is large, some problems have occurred in applications to natural populations. Using Schaefer's data on sockeye salmon (*Oncorhynchus nerka*), Darroch showed that recapture rate estimates for some strata exceeded 1, the upper limit of their domain. He attributed the problem to sampling error and unequal rates of natural mortality among strata but found no satisfactory solution. Similar problems were encountered by Arnason (1972, 1973), who extended the mark–recapture method to accommodate multiple recaptures. Using simulation experiments, Arnason (1972) showed that some combinations of the underlying rates of migration and recapture produced recapture rate estimates that were either less than 0 or greater than 1, again outside the domain on which they are defined. Arnason provided guidelines, based on simulation studies, for minimum numbers of

recaptures required to plan or evaluate mark–recapture experiments with geographically stratified populations. The guidelines were reported to reduce bias and improve precision of the parameter estimates but did not eliminate the possibility of undefined parameter estimates.

We here describe simulation studies that clarify the origin of estimation problems that can occur in mark–recapture experiments with stratified populations. Then, using Schaefer's data on sockeye salmon, we show that symmetric reductions of the matrix of stratified recaptures can reduce the bias and improve the precision of the stratum-specific estimates of recapture rates and abundances. The advantage of this approach, which was mentioned by Darroch but not used in his analysis of the salmon data, is that unequal rates of natural mortality among strata need not be assumed to obtain unbiased and precise estimates of recapture rates and abundances.

Methods

Theory

The formal statistical model for estimating recapture and migration rates in stratified populations was developed by Darroch (1961). Here, we consider mark–recapture experiments in which fish are tagged and released in s distinct strata and recovered subsequently from s strata, which may or may not be identical with those used to distinguish the fish at release. Following Darroch, we assume that all fish behave independently with regard to migration and recapture and that the population is closed, i.e. mortality occurs only during loss on capture. (Later, we consider the consequences of relaxing the second assumption and allowing natural mortality to vary among strata). The expected number of fish that are tagged and released in stratum i and recovered from stratum j is

$$(1) \quad E(c_{ij}) = a_i \, \Theta_{ij} \, p_j$$

in which a_i is the number of fish tagged and released in stratum i, Θ_{ij} is the probability that fish move from stratum i to stratum j during the experiment, and p_j is the probability that a fish in stratum j will be recovered. Fish may be recovered by a planned, fishery-independent survey or by fishermen that sample the strata opportunistically. In the former case, population abundance can be estimated from the recoveries of tagged and untagged fish in each stratum. In the latter case, stratum-specific rates of exploitation by fishermen are estimated by the stratum-specific recapture rates.

Darroch showed that the maximum likelihood estimator of the probability of recapturing an individual in stratum i, given that the individual is in that stratum at the time of sampling, is

$$(2) \quad \hat{\rho} = C^{-1} \, a.$$

The matrix C is the s by s matrix of recaptures $[(c_{ij})]$, a is the s by 1 column vector of releases $[(a_i)]$, and $\hat{\rho}$ is an s by 1 column vector of reciprocals of the conditional recapture probabilities $[(\hat{p}_i)^{-1}]$. The probability that individuals move from stratum i to stratum j may be estimated from

$$(3) \quad \hat{\Theta}_{ij} = a_i^{-1} \, c_{ij} \, \hat{\rho}_j.$$

An estimate of the variance–covariance matrix of $\hat{\rho}$ is

$$(4) \quad \hat{\Sigma} = D_{\hat{\rho}} \, \hat{\Theta}^{-1} \, D_{\hat{\mu}} \, D_a^{-1} \, \hat{\Theta}'^{-1} \, D_{\hat{\rho}}$$

in which D_a and $D_{\hat{\rho}}$ are diagonal matrices with the elements of a and $\hat{\rho}$, respectively, on the main diagonal and with zeroes elsewhere. $D_{\hat{\mu}}$ is also a diagonal matrix; its nonzero elements are

$$\hat{\mu}_i = \sum_j (\hat{\Theta}_{ij} \, \hat{\rho}_j) - 1.$$

The standard error of each element of $\hat{\rho}$ is estimated from $\hat{\rho}$ and the diagonal elements of $\hat{\Sigma}$ $[V(\hat{\rho}_i))]$ using

$$(5) \quad \mathrm{SE}(\hat{p}_i) = \hat{\rho}_i^{-2} \, (V(\hat{\rho}_i))^{1/2}.$$

The recapture matrix C must be nonsingular to estimate ρ, Θ, and Σ (equation (2)). Near-singular recapture matrices, however, may occur under various types of experimental conditions. To identify the possibilities, we derive the expected value of the determinant of C as follows. First, we note that the expected value of C is

$$(6) \quad E(C) = D_a \, \Theta \, D_p.$$

The matrix D_p has the elements of p on the main diagonal and zeroes elsewhere. The expected value of the determinant of C is therefore

$$(7) \quad E(|C|) = |D_a| \, |\Theta| \, |D_p| = |\Theta| \prod_i a_i \, p_i.$$

Experimental conditions that produce near-singular recapture matrices (i.e. $E(|C|) \sim 0$) are evident from equation (7). The determinant of Θ equals zero exactly when migration rates to the recovery strata are the same for at least two release strata, i.e. $\Theta_{ij} = \Theta_{kj}$ for $i \neq k$, $j = 1$ to s. In this situation at least one row of the recapture matrix is expected to be a linear combination of another. Darroch showed that a χ^2 goodness-of-fit test may be used to determine whether the rows of C are homogeneous. Equation (7) also shows that the incidence of near-singular recapture matrices can be reduced by increasing the numbers of releases or by increasing recapture rates. These results confirm Arnason's (1972) recommendations, at least qualitatively, for increasing numbers of releases to improve the accuracy and precision of recapture rate estimates. Unfortunately the results offer little insight into how the estimates become undefined (i.e. $\hat{p}_j < 0$, or $\hat{p}_j > 1$) and what can be done when one is confronted with such estimates.

Simulations

We addressed these problems by conducting Monte Carlo simulation experiments in which we specified underlying values for a, p, and Θ. For each set of underlying values, we approximated the sampling distributions of the number of tag recoveries and of the estimated rates of movement and recapture. Movement of fish between strata and recaptures within strata were treated as sequential, stochastic events. In that way, our simulations satisfied the underlying assumptions of Darroch's estimators for the rates of movement and recapture.

Our simulated experiments included movements of fish between two strata. The number of fish that moved from stratum i to stratum j in any single experiment was computed from the outcome of a_i independent trials in which random values were drawn from a uniform distribution on the [0,1] interval. Each trial that resulted in a random number less than or equal to Θ_{ij} represented movement of an individual from stratum i to j. Individual outcomes of such trials therefore define a Bernoulli-distributed random variable with parameter Θ_{ij} (Mood et al. 1974). The total number of animals that moved from stra-

tum i to j, k_{ij}, was the sum of such outcomes; the remaining $a_i - k_{ij}$ animals stayed within release stratum i. We computed recapture frequencies c_{ii} and c_{ij} similarly, using the underlying probabilities of recapture, p_i and p_j, respectively. In this computation, however, the numbers of independent trials were random variables because they comprised variable outcomes of the simulated movements from stratum i to j. The frequency of recaptures of animals that moved from stratum i to j was computed from the sum of k_{ij} Bernoulli trials, each with probability p_j. The frequency of recaptures of animals that remained within stratum i was computed from the sum of $a_i - k_{ij}$ Bernoulli trials, each with probability p_i.

Each tagging experiment was simulated 1000 times to approximate the sampling distributions of recapture frequencies c_{ij}. Equations (2)–(5) were used to estimate p_i and SE(p_i) for each experiment and thereby approximate their sampling distributions. To gain insight into the problems associated with parameter estimation, we examined the distributions of $\hat{p}_i$ and SE($\hat{p}_i$) for a range of underlying values of a, p, and Θ. Here, we illustrate our general findings with the following set of values for p and Θ: $p_1 = 0.5$, $p_2 = 0.5$, $\Theta_{11} = 0.9$, $\Theta_{12} = 0.1$, $\Theta_{21} = 0.8$, $\Theta_{22} = 0.2$. This set of parameters produced recovery matrices that were either singular, near-singular, or nonsingular depending on the number of fish released from each of the two strata, which we varied from 100 to 5000.

An Example with Sockeye Salmon

We used temporally stratified recoveries of sockeye salmon (Schaefer 1951) to illustrate how symmetric reductions of the recapture matrix can reduce bias and improve precision of the stratum-specific estimates of recapture rate. During a period of 8 wk, Schaefer marked adult salmon that were migrating upstream toward spawning grounds. The fish were tagged according to the week of capture as they passed a certain location in the river. The tagged fish were subsequently recovered on the spawning grounds over a period of 9 wk. Release and recovery periods did not overlap.

Few recoveries of "early-" or "late-spawning" salmon were made. We therefore followed Darroch's recommendation and pooled the first 3 and last 3 wk of tagging into single strata and the first 3 and last 4 wk of recovery into single strata. The resulting 4 by 4 matrix of recoveries is given in Table 1. To compare the effects of alternative stratifications of the tagging data, we estimated stratum-specific recapture rates and their standard errors using equations (2)–(5). In addition to the 4 by 3 stratification that Darroch recommended (Table 1), we examined two alternatives. We combined the second and third rows (and columns) of the 4 by 4 recapture matrix to form a 3 by 3 stratification (Table 1) because salmon tagged during the fourth and fifth weeks were recovered in nearly the same proportions. We intended to remove linear dependence among the rows of C and improve the accuracy and precision of our estimates of recapture rate. We also analyzed a 2 by 2 stratification of the data in which the first two and the last two rows (and columns) of the 4 by 4 recapture matrix were combined (Table 1). This stratification was intended to compare the rates of recapture of early- and late-spawning salmon.

TABLE 1. Various stratifications of tag releases and tag recoveries of adult sockeye salmon (Schaefer 1951). Salmon were recovered on the spawning grounds during a 9-wk period that followed 8 wk of tagging at a downstream location.

4 by 4 Stratification

Week of tagging	No. of recaptures c_{ij} at week of spawning and recovery: 1–3	4	5	6–9	No. of releases a_i
1–3	59	24	5	2	484
4	34	79	52	15	695
5	11	67	77	28	773
6–8	0	14	25	28	399

4 by 3 Stratification

Week of tagging	No. of recaptures c_{ij} at week of spawning and recovery: 1–3	4	5–9	No. of releases a_i
1–3	59	24	7	484
4	34	79	67	695
5	11	67	105	773
6–8	0	14	53	399

3 by 3 Stratification

Week of tagging	No. of recaptures c_{ij} at week of spawning and recovery: 1–3	4–5	6–9	No. of releases a_i
1–3	59	29	2	484
4–5	45	275	43	1468
6–8	0	39	28	399

2 by 2 Stratification

Week of tagging	No. of recaptures c_{ij} at week of spawning and recovery: 1–4	5–9	No. of releases a_i
1–4	196	74	1179
5–8	92	158	1172

Results

From the simulated tagging experiments, we discovered that undefined estimates of recapture rates were associated with recapture data that produce extremely "flat" likelihood surfaces and imprecise estimates of p. To show this, we plotted separately the sampling distributions of SE($\hat{p}_2$) associated with different ranges of $\hat{p}_2$ (Fig. 1). High values of SE ($\hat{p}_2$) indicate that the magnitude of the likelihood function remains relatively constant over a wide range of values for $\hat{p}_2$. When 100 fish were released from each stratum, $\hat{p}_2$ was negative in 22.3% of the simulated experiments and $\hat{p}_2$ exceeded 1 in 9.8% of the simulated experiments. The estimates of SE (p_2) in these experiments generally exceeded estimates of SE(p_2) computed for experiments where $\hat{p}_2$ was in the [0,1] interval; differences in SE ($\hat{p}_2$) of one or two orders of magnitude were not uncommon. Such large differences were usually associated with extraordinarily biased estimates of p_2, which ranged from -5.68 to 5.90. Although increases in the number of fish released in each stratum reduced the incidence of undefined $\hat{p}_2$, the estimates were always imprecise.

Our simulated tagging experiments also showed that estimates of recapture rates within the [0,1] interval are not necessarily unbiased. When only 100 fish were released from each of the two strata, 71% of the estimates of p_2 were less than its underlying value of 0.5 (Fig. 2). Bias of the p_2 estimates

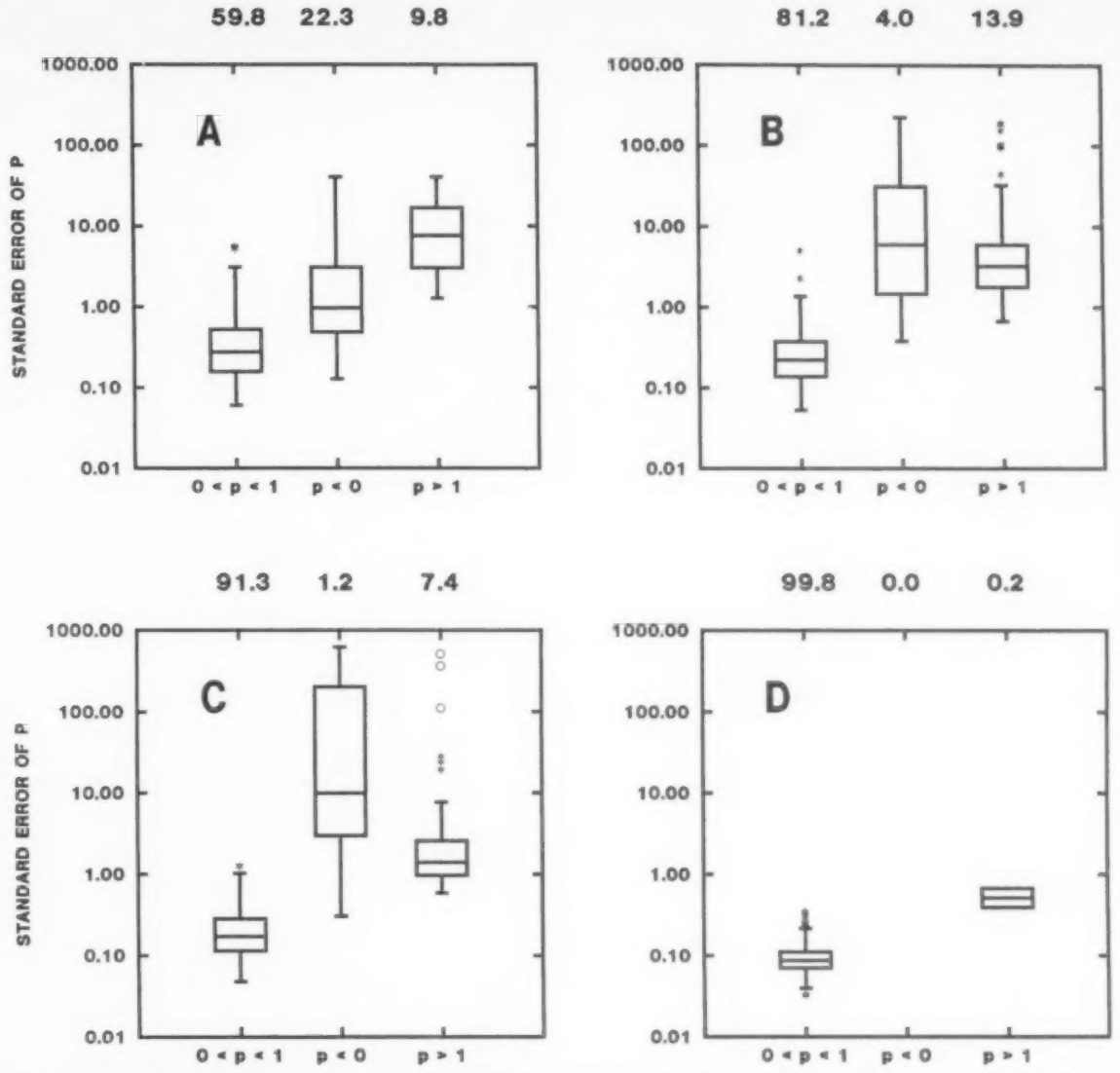


FIG. 1. Box plots (Wilkinson 1988) of simulated sampling distributions of SE($\hat{p}_2$). Underlying values of Θ and p were selected to produce near-singular recapture matrices when few fish were released from the two strata (see text). Each distribution of SE ($\hat{p}_2$) is stratified by the range of values estimated for p_2. Percent of cases associated with each range is indicated above each box plot. Percent of cases with singular recapture matrices decreased with increases in the number of fish released in each stratum: (A) 8.1% with 100 releases, (B) 0.9% with 500 releases, (C) 0.1% with 1000 releases, and (D) none with 5000 releases.

decreased when more tagged fish were released in each stratum. In our experiments, estimates of p_2 were approximately unbiased when 5000 fish were released from each stratum.

In the tagging experiment with sockeye salmon, we determined that the 3 by 3 and the 2 by 2 stratifications of the recapture data provided more precise estimates of recapture rates than the 4 by 4 or the 4 by 3 stratification (Fig. 3). For the 4 by 4 stratification, the estimated probability of recapture during the fourth week of spawning was 1.94 (SE = 13.2). The 4 by 3 stratification helped estimate this probability within the [0,1] interval, but the variance of the estimate remained high (SE = 1.88) relative to the variability of recapture rates estimated for the other recovery periods. The 3 by 3 and the 2 by 2 stratifications of the data provided estimates of recapture rates that were all within the [0,1] interval and were all relatively precise (SEs ranged from 11 to 25% of the estimates).

Discussion

Although movement of animals among geographic areas is widely recognized as an important problem in population dynamics, the theoretical work of Darroch (1961) has received little attention until recently. Arnason (1972, 1973) extended Darroch's work to address multiple recaptures.Sibert (1984) developed a system of first-order differential equations to describe patterns of tag recaptures in two fisheries. Hilborn

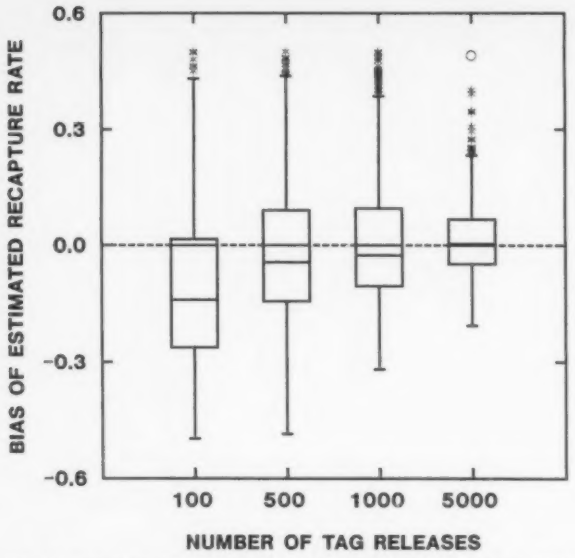


FIG. 2. Box plots (Wilkinson 1988) of sampling distributions of the bias of $\hat{p}_2$ (i.e. $\hat{p}_2 - p_2$) approximated by Monte Carlo simulations conducted over a range of numbers of tag releases. Underlying values of Θ and p were selected to produce near-singular recapture matrices when few fish were released from the two strata (see text). Box plots of bias do not include $\hat{p}_2$ estimates that exceeded 1 or were negative.

(1990) generalized Sibert's approach to include s areas and formulated the distribution of tag recoveries as a Poisson distribution. He then applied maximum likelihood theory to estimate the rates of migration and recapture.

Both Darroch's and Hilborn's models apply to terminal recaptures. Consequently, neither can distinguish "backtracking" of fish, which occurs when fish migrate from one stratum to another and then return to the stratum of release. Therefore, rates of migration estimated from stratified tagging experiments actually correspond to *net* rates of exchange between strata. In experiments with migratory fish populations, the net rates of movement between strata will generally depend on the size of the strata and on the duration of the experiment. Both aspects of an experiment's design must therefore be considered to be able to interpret the migration rate estimates in a biological context.

Hilborn (1990) asserted that the statistical complexity of analysis of stratified tagging data should not be intimidating because parameters can easily be estimated by numerical optimization of an appropriate likelihood function. This is true provided the likelihood function has a well-defined maximum within the range of feasible parameter values. Near-singular recapture matrices, which can arise from particular combinations of the underlying parameters or from sampling variability, produce "flat" likelihood surfaces. Such surfaces imply low precision for parameter estimates and may imply bias as well. Our simulated tagging experiments illustrated that even simple experiments with two strata can produce highly biased estimates. In addition to the obviously biased recapture probabilities estimated outside the [0,1] interval, we obtained estimates within the [0,1] interval that were more precise but not necessarily accurate (Fig. 1 and 2). The estimates were biased downward, and this proved to be generally true whenever the simulated movements of fish among strata produced near-singular recapture matrices. Increases in the number of fish released from the two strata were required to reduce the variance and bias of the estimated recapture probabilities.

Evaluation of Alternative Models

Our analysis of Schaefer's data (Table 1) showed that reductions in the number of strata could decrease the variability of recapture rate estimates. Darroch showed that recapture rates can be estimated when reductions in strata result in either symmetric (s by s) or asymmetric (s by t, where $s > t$) recapture matrices. When $s > t$, the number of parameters to be estimated is reduced from $st - 1 + t$ to st by imposing $t - 1$ constraints on the rates of natural mortality among strata. The 4 by 3 stratification illustrated by Darroch produced feasible but imprecise estimates of recapture probabilities (Fig. 3). In addition, Darroch's estimate of the SE of the rates of natural mortality indicated that the assumed stratum-specific differences in these rates were not significant ($p > 0.10$).

The appropriate number of strata for release and recaptures should be the maximum number that provides estimates of recapture probabilities on the [0,1] interval with standard errors within predetermined acceptable levels. Unfortunately, rigorous statistical procedures for comparing the goodness-of-fit of alternative models have not been developed. Darroch used the likelihood-ratio method to test specific hypotheses related to homogeneity of the rows of the recapture matrix. This approach permits specific models to be compared and provides guidance on potential changes in stratification, but it does not indicate how well the data fit a specific model. The χ^2 goodness-of-fit tests commonly applied to band recovery models (Brownie et al. 1985) cannot be applied to Darroch's model because the number of parameters to be estimated equals the numbers of elements in the recovery matrix. If tagged fish can be identified uniquely and sufficient numbers are released, cross-validation techniques (Nichols and Hines 1983) can be applied to test the suitability of a particular model. For example, the recoveries associated with a particular subset of the releases in each stratum could be used to estimate Θ and p. These estimates could then be used to compute a matrix of predicted recoveries $\hat{C}$ given the remaining stratum-specific releases not used to estimate Θ and p, i.e. $\hat{C} = D_{a^*} \hat{\Theta} D_{\hat{p}}$. A χ^2 test could then be applied to test whether the predicted matrix of recoveries $\hat{C}$ is significantly different than the observed matrix of recoveries not used to estimate Θ and p. Absence of a significant difference would indicate that the recapture data fit Darroch's model well. Unfortunately, Schaefer's paper did not contain sufficient detail for us to attempt this type of cross-validation. In general, partitioning of the data for the purposes of cross-validation may increase the sampling variability of the parameter estimates, but the ability to test whether Darroch's model and the number of strata used is appropriate is probably worth the trade-off.

Effects of Natural Mortality, Tag Loss, Tagging Mortality, and Incomplete Reporting of Recoveries

The results of tagging experiments are potentially influenced by natural mortality, tag loss, mortality associated with tag application, and incomplete reporting of tag recoveries. The stratified tagging experiments that we have described cannot provide independent estimates of these factors, but it is instructive to evaluate their influence on Darroch's recapture rate estimators.

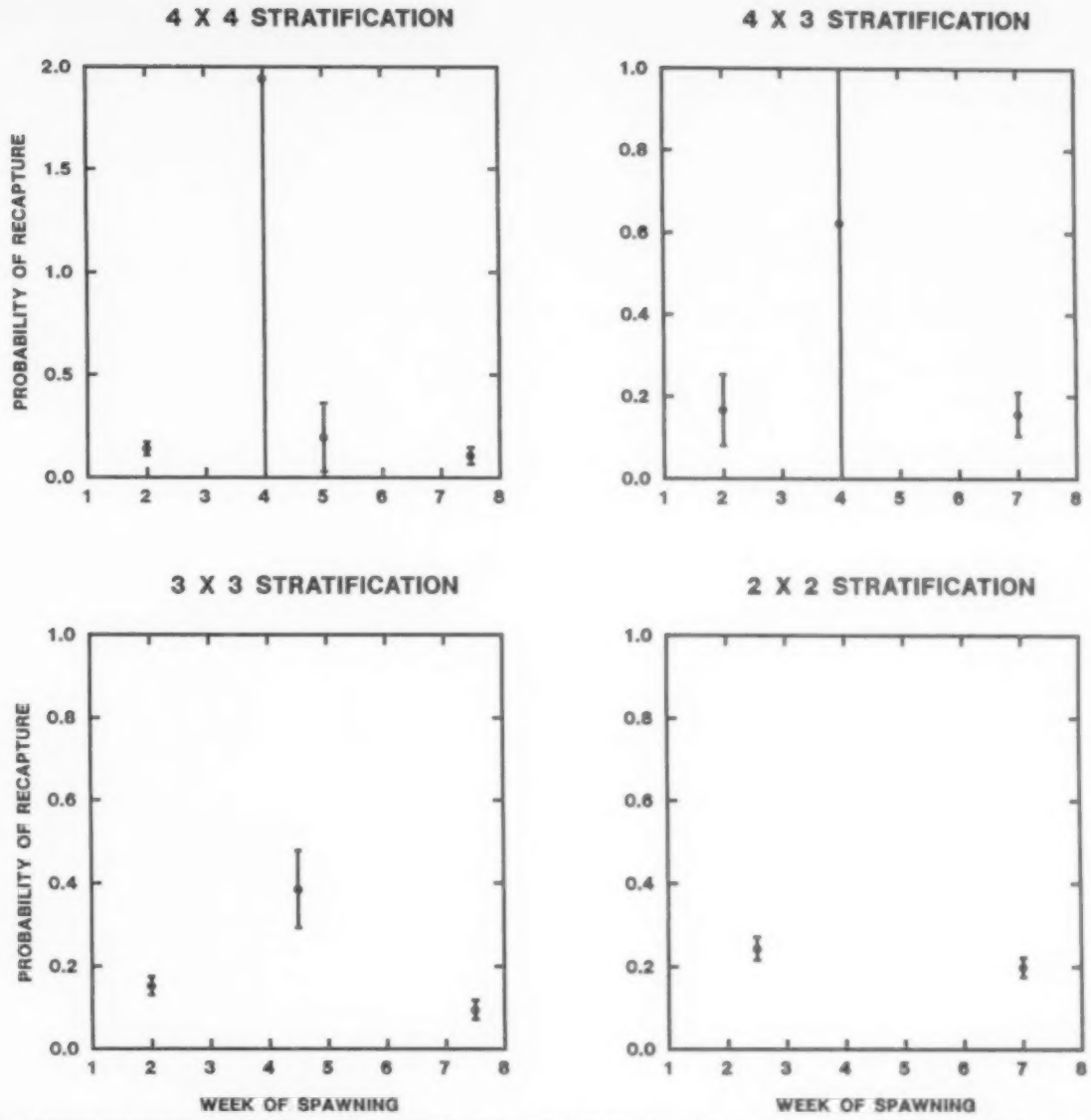


FIG. 3. Estimates of recapture probabilities for the four stratifications of Schaefer's salmon data presented in Table 1. Error bars indicate ± 1 SE.

Darroch assumed that all tagged fish survive between release and recapture, which implies

$$\sum_j \Theta_{ij} = 1, \qquad \text{for all } i.$$

In matrix notation this equation is

(8) $\Theta\, 1 = 1$

in which 1 is an s by 1 unit vector. In tagging experiments with fish populations, some fish may die or lose their tags before being recaptured; others are recovered but not reported. Darroch showed that stratum-specific rates of natural mortality could be incorporated into his model as follows:

(9) $\Theta\, 1 = D_\phi\, 1.$

Each diagonal element of the s by s diagonal matrix D_ϕ is the probability that a fish released in stratum i survives natural mortality to reach its stratum of recapture. We can extend Darroch's model by defining diagonal matrices that contain stratum-specific conditional probabilities for tag retention D_γ, survival of tag application D_δ, and reporting of tag recoveries D_β. Given these assumptions, the expected matrix of recaptures is

(10) $E(C) = D_\gamma D_\delta D_a \Theta D_\beta D_\rho.$

Substituting equation (9) into (10) and rearranging yields an estimator for the vector of reciprocals of conditional recapture probabilities:

(11) $\hat{\rho} = D_\beta C^{-1} D_\gamma D_\delta D_\phi a.$

Thus, mortality and tag loss influence recapture rate estimates by reducing the effective number of releases in each stratum. Incomplete reporting of tag recoveries, on the other hand, affects the estimate of recapture rate in a stratum by reducing

the number of recoveries in that stratum, regardless of where fish were released. Additional experiments would be required to estimate β_i, ϕ_i, γ_i, or δ_i independent of p_i. However, equation (11) can still be used to examine the sensitivity of stratum-specific recapture rates to assumed differences in these parameters. Because these parameters are generally important in tagging experiments with fish populations, such sensitivity analyses could prove valuable to assessments of stratum-specific rates of exploitation imposed by commercial or recreational fisheries (Vetter 1988).

Acknowledgments

We thank James D. Nichols, Carl J. Schwarz, and an anonymous referee for their thoughtful reviews of the manuscript. Our work was supported in part by the Emergency Striped Bass Research Study, an amendment to the Anadromous Fish Conservation Act (Public Law 96-118).

References

ARNASON, A. N. 1972. Parameter estimates from mark–recapture experiments on two populations subject to migration and death. Res. Popul. Ecol. 13: 97–113.

1973. The estimation of population size, migration rates and survival in a stratified population. Res. Popul. Ecol. 15: 1–8.

BROWNIE, C., D. R. ANDERSON, K. P. BURNHAM, AND D. S. ROBSON. 1985. Statistical inference from band recovery data — a handbook. 2nd ed. U.S. Fish Wildl. Serv. Resour. Publ. No. 156, Washington, DC. 305 p.

DARROCH, J. N. 1961. The two-sample capture–recapture census when tagging and sampling are stratified. Biometrika 48: 241–260.

HILBORN, R. 1990. Determination of fish movement patterns from tag recoveries using maximum likelihood estimators. Can. J. Fish. Aquat. Sci. 47: 635–643.

MOOD, A. M., F. A. GRAYBILL, AND D. C. BOES. 1974. Introduction to the theory of statistics. 3rd ed. McGraw-Hill, New York, NY. 564 p.

NICHOLS, J. D., AND J. E. HINES. 1983. The relationship between harvest and survival rates of mallards: a straightforward approach with partitioned data sets. J. Wildl. Manage. 47: 334–348.

SCHAEFER, M. B. 1951. Estimation of size of animal populations by marking experiments. Fish. Bull. U.S. 52: 191–203.

SIBERT, J. R. 1984. A two-fishery tag attrition model for the analysis of mortality, recruitment, and fishery interaction. Tuna and Billfish Assessment Programme, South Pacific Commission, Noumea, New Caledonia, Tech. Rep. No. 13: 27 p.

VETTER, E. F. 1988. Estimation of natural mortality in fish stocks: a review. Fish. Bull. U.S. 86: 25–43.

WILKINSON, L. 1988. SYGRAPH. SYSTAT, Inc., Evanston, IL. 922 p.

Habitat Partitioning and Contaminant Exposure in Cyprinids

Craig E. Hebert and G. D. Haffner

Great Lakes Institute, Biological Sciences, University of Windsor, Windsor, Ont. N9B 3P4, Canada

Hebert, C. E., and G. D. Haffner. 1991. Habitat partitioning and contaminant exposure in cyprinids. Can. J. Fish. Aquat. Sci. 48: 261–266.

Four species of forage fish from the Huron–Erie corridor — *Labidesthes sicculus*, a surface feeder; *Notropis atherinoides*, a facultative surface feeder; *Notropis hudsonius*, a facultative benthivore; and *Pimephales notatus*, a benthivore — were found to have significantly different levels of organochlorine contaminants with high 1-octanol/water partition coefficients (K_{ow}). Chemical levels were greatest in *P. notatus*, reflecting its greater exposure to contaminated sediments. The interspecific differences observed indicate that habitat partitioning is a major factor regulating contaminant levels in these forage fish species. These interspecific differences in contaminant levels have important implications for fugacity models which would predict that these differences should not exist. Although chemical and physiological parameters may determine which contaminants have the potential to bioaccumulate, it is the regulation of exposure through ecological processes that will determine the degree to which that potential is realized.

Des concentrations significativement différentes de contaminants organochlorés à K_{ow} (coefficient de partage 1-octanol/eau) élevé ont été observées dans quatre espèces de cyprinidés du corridor des lacs Huron et Érié : *Labidesthes sicculus*, espèces se nourrissant en surface; *Notropis atherinoides*, se nourrissant facultativement à la surface; *Notropis hudsonius*, un benthivore facultatif; et *Pimephales notatus*, un benthivore. Les concentrations étaient plus élevées chez *P. notatus*, ce qui indique que cette espèce est davantage exposée à ces substances. Les différences interspécifiques observées révèlent que les zones de l'habitat exploitées par ces poissons n'ont pas toutes le même pouvoir de contamination; il s'agit là d'un facteur clé dans la régulation des taux de contamination de ces espèces de poisson fourrage. Ces différences interspécifiques des taux de contamination ont des conséquences importantes pour les modèles de fugacité qui prédiraient que ces différences n'existeraient pas. Certains paramètres chimiques et physiologiques peuvent permettre de déterminer quels contaminants ont un potentiel de bioaccumulation, mais ce sont les processus écologiques régulant l'exposition aux substances toxiques qui détermine le degré auquel ce potentiel se réalise.

Received October 17, 1989
Accepted August 10, 1990
(JA342)

Reçu le 17 octobre 1989
Accepté le 10 août 1990

Debate exists concerning the role that biomagnification plays in regulating contaminant distribution in aquatic ecosystems. The thermodynamic approach, based on fugacity models, predicts that organisms in equilibrium with their environment should show similar levels of contaminants in their lipid fractions, regardless of their trophic status or habitat utilization (Connolly and Pederson 1988). An alternate model based on food web dynamics incorporates the process of biomagnification, thereby allowing for interspecific differences in lipid-normalized contaminant levels (Thomann and Connolly 1984).

The fugacity approach divides the environment into phases such as biota, sediment, suspended solids, water, and air. If transfer of a chemical is to occur passively among these compartments, then a gradient in chemical potential, or fugacity, must exist. At equilibrium, the fugacity of all phases is assumed to be equal with no net transfer of contaminants. In such a balanced aquatic ecosystem one would not predict any differences in lipid-normalized contaminant levels among aquatic organisms.

When differences in contaminant levels have been observed between trophic levels, these differences have been dismissed as being relatively small and only occurring for a few very hydrophobic compounds (Thomann and Connolly 1984). Such differences have also been attributed to nonequilibrium conditions which assume that organisms are moving towards equifugacity, possibly at different rates, but have not yet reached that state. Nonequilibrium dynamics, for example, would also result from the slow uptake rates of highly hydrophobic compounds compared with organism growth rates. Therefore, large species at higher trophic levels would have the greatest chemical body burdens, not because feeding provides additional exposure to the chemical but because exposure duration would be greatest and chemical elimination would be slowest for these species.

Other work has suggested that an alternate model based on food web dynamics can better explain the distribution of contaminants in aquatic ecosystems. Borgmann and Whittle (1983) illustrated that larger organisms had higher contaminant levels than smaller organisms, but as their data were not lipid normalized, it is not possible to demonstrate biomagnification. Their results did suggest, however, that biomagnification could occur. Thomann and Connolly (1984) developed an age-dependent food chain model for polychlorinated biphenyls in the Lake Michigan food chain and concluded that the major route of PCB uptake was from food. This mechanism was further substantiated by Oliver and Niimi (1988) who demonstrated the importance of "trophodynamics" in contaminant partitioning in the Lake Ontario ecosystem. Similar observations have been made by Connolly and Pederson (1988) and

Gobas et al. (1988), resulting in considerable evidence that food resources play an important role in determining the distribution and levels of selected organic contaminants in aquatic food webs.

If food is an important exposure route, then differences in resource utilization among species can play an important role in regulating their contaminant body burdens (Flint et al. 1988). To further resolve the factors regulating contaminant levels in aquatic organisms, we examined contaminant levels in forage fish. Within this component of the food web, benthic, pelagic, and allochthonous food webs are differentially utilized by philopatric species of similar size and age. Should chemical properties, related to the bioconcentration potential of contaminants, be singularly important (Neely et al. 1974; Veith et al. 1979), one would expect negligible interspecific differences amongst these organisms. The importance of chemical hydrophobicity in determining contaminant levels in fish has been demonstrated (Johnson et al. 1988). Similarly, other chemical factors such as aqueous solubility, which regulates the bioavailability of a compound, should also result in equivalent body burdens. Should, however, differences in lipid-normalized contaminant levels be observed, other regulating factors such as metabolic differences and/or differences in exposure dynamics would need to be examined.

There is, however, little information on the relative importance of ecological factors in determining both contaminant levels and distribution in aquatic food webs. As a result, different biomonitors are often assumed to integrate system changes in contaminant levels in a similar manner. This assumption might not be justified for organisms that differ either ecologically or metabolically.

Cyprinid fish populations and ecologically similar species provide an opportunity to test this assumption. *Labidesthes sicculus* is primarily a surface-feeding species whose diet consists primarily of small flying insects and some cladocerans (Scott and Crossman 1973). *Notropis atherinoides* is a facultative surface feeder whose diet consists primarily of zooplankton (Scott and Crossman 1973). *Notropis hudsonius* consumes a wide variety of organisms including cladocerans, aquatic insect larvae, and algae. *Pimephales notatus* subsists almost entirely on chironomid larvae and organic detritus from the bottom (Scott and Crossman 1973). These species represent an integration of allochthonous, pelagic, and benthic food webs. In a study by Muth and Busch (1989) there was little dietary overlap between *N. hudsonius* and *N. atherinoides*, with *N. atherinoides* mainly feeding on cladocerans. In this study, the relative importance of chemical and ecological factors in determining contaminant burdens in cyprinids is examined.

Materials and Methods

Fish were collected from two sampling locations (Fig. 1). The first site was located in the St. Clair River approximately 1.5 km south of Sarnia's Chemical Valley and was close to important sources of chemical contamination on the Canadian side of the river (Kauss and Hamdy 1985). Water was shallow (1–1.5 m) and well mixed. Sediment consisted of a mixture of silt and coarse sand. A second site, located in the Trenton Channel of the Detroit River near the southern tip of Grosse Ile, was chosen because it is also heavily contaminated (Kauss and Hamdy 1985).

The St. Clair River site was sampled during August and September 1987. Young-of-the-year of the four fish species were

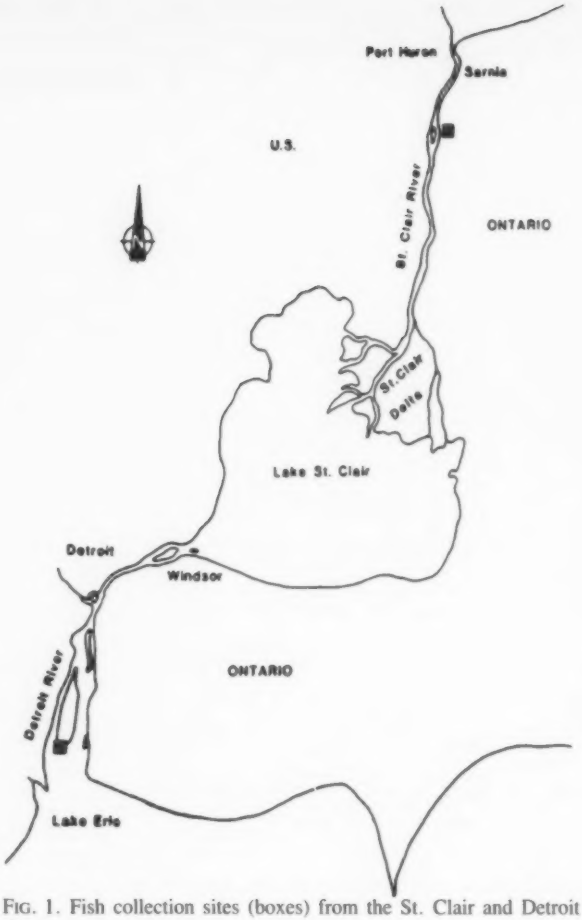


FIG. 1. Fish collection sites (boxes) from the St. Clair and Detroit rivers.

caught in nearshore waters using a 10-m, 0.6-cm-mesh bagseine. Immature fish were collected to minimize intersexual differences. Fish were measured (total length), grouped according to length, and immediately wrapped in hexane-rinsed aluminum foil. Samples were kept frozen at −20°C until they were analyzed. This site was sampled again in September 1988 and at this time both fish and sediment samples were collected. The top 3 cm of sediment was collected and placed in hexane-rinsed amber glass jars. Jars were also stored at −20°C.

During October of 1988, *L. sicculus* and *P. notatus* were collected from the Detroit River site. Sediment samples were also collected as previously described.

The compounds we examined varied over two orders of magnitude in their 1-octanol/water partition coefficients (K_{ow}). Pentachlorobenzene (log K_{ow} = 5.2), hexachlorobenzene (5.5), octachlorostyrene (6.3), and PCB congeners 101 (6.38) and 180 (7.36) were examined at both sites. In addition, at the Detroit River site, *p,p'*-DDE (log K_{ow} = 5.69) and PCB congeners 31 (5.7), 52 (5.84), 87 (6.29), 118 (6.74), 138 (6.83), and 153 (6.92) were examined. Fish from the St. Clair River had low levels of PCBs; therefore, we examined the wide range of congeners only in 1988.

Whole fish were analyzed according to extraction and cleanup protocols developed by the Canadian Wildlife Service

(1982). All samples consisted of approximately five individuals and the composite wet weight was not less than 3 g.

Sediment samples were analyzed using Soxhlet procedures. For all samples, only the first fraction from the Florisil column was collected. This fraction contained chlorobenzenes, octachlorostyrene, and various PCB congeners. Recovery efficiencies for this method were 87% for QCB, 89% for HCB, and 91% for OCS. Recovery efficiencies for the PCB congeners were 90%. Reported values were not corrected for recovery efficiencies. To determine if the existing gut content could affect body burden, gutted fish were analyzed and compared with body burdens of whole-fish samples. Lipid determinations were made on each sample. The organic carbon content of each of the sediment samples was determined using loss on ignition.

The samples were injected into a Hewlett-Packard model 5790A GC-ECD fitted with a 30 m × 0.25 mm DB-5 capillary column (J + W Scientific). Standards (QCB and HCB, Chemservice; OCS, Health and Welfare Canada; PCB congeners, Environment Canada) and a solvent blank, which had undergone the entire isolation procedure, accompanied each set of six samples. For QCB, HCB, and OCS the limit of quantification was 0.1 μg/kg. For the PCB congeners the limit of quantification was 0.2 μg/kg.

Rudimentary gut contents analysis was completed for *P. notatus* and *L. sicculus* to verify resource partitioning. Three food types were included: chironomid larvae (benthic), bosmina (pelagic), and chironomid adults (allochthonous). Other food items were not included.

Bioaccumulation factors (BAFs) were estimated for each of the species at both sites during 1988 to determine the relationship among contaminant levels in fish and sediment and K_{ow}. BAFs were calculated by dividing the lipid-normalized contaminant levels in the fish by the organic carbon normalized contaminant levels in the sediment.

Results

Considerable interspecific compartmentalization of the contaminants occurs in fish from the St. Clair River (Fig. 2). Significant interspecific differences in $\log_{10}$-transformed, lipid-normalized contaminant levels were detected using ANOVA ($p < 0.05$) followed by Tukey's test ($p < 0.05$). A similar pattern of chemical compartmentalization for *L. sicculus* and *P. notatus* is seen in the Detroit River (Fig. 3). Significant interspecific differences in $\log_{10}$-transformed, lipid-normalized contaminant levels were detected using Student's *t*-test ($p < 0.05$). In both the St. Clair and Detroit rivers, *P. notatus* generally has the highest mean contaminant levels and *L. sicculus* the lowest. In the St. Clair River, mean contaminant levels in *P. notatus* are followed by *N. hudsonius* and then by *N. atherinoides*. It is also evident (Fig. 2 and 3) that significant interspecific differences are only seen for the higher K_{ow} compounds. No significant differences in length or lipid content were observed among the species at the St. Clair River site (ANOVA, $p > 0.1$) or at the Detroit River site (*t*-test, $p > 0.1$). Therefore, contaminant distribution in these species is not a function of allometric differences. Contaminant burdens in gutted *P. notatus* are not significantly different from comparable samples consisting of whole fish (*t*-test, $p > 0.1$). Analysis of gut contents revealed that the diet of *L. sicculus* consists primarily of prey associated with surface waters. *Pimephales notatus*, however, feeds primarily on benthic organisms (Fig. 4).

Figure 5A shows a plot of log BAF versus log K_{ow} for three of the four species at the St. Clair River site. These fish were collected in September 1988 along with sediment from that site. *Notropis atherinoides* was not present in the collections. There is a linear relationship between log BAF and log K_{ow} for the three species (*L. sicculus*, $r^2 = 0.81$; *N. hudsonius*, $r^2 = 0.88$; *P. notatus*, $r^2 = 0.93$). ANOVA indicates that the slopes of these lines are not significantly different ($p > 0.1$), but the intercepts differ ($p < 0.05$). *Pimephales notatus* and *N. hudsonius* have significantly greater BAFs than *L. sicculus*. At the Detroit River site (Fig. 5B), a linear relationship exists between log BAF and log K_{ow} for both species (*L. sicculus*, $r^2 = 0.45$; *P. notatus*, $r^2 = 0.64$). ANOVA indicates that there is no sig-

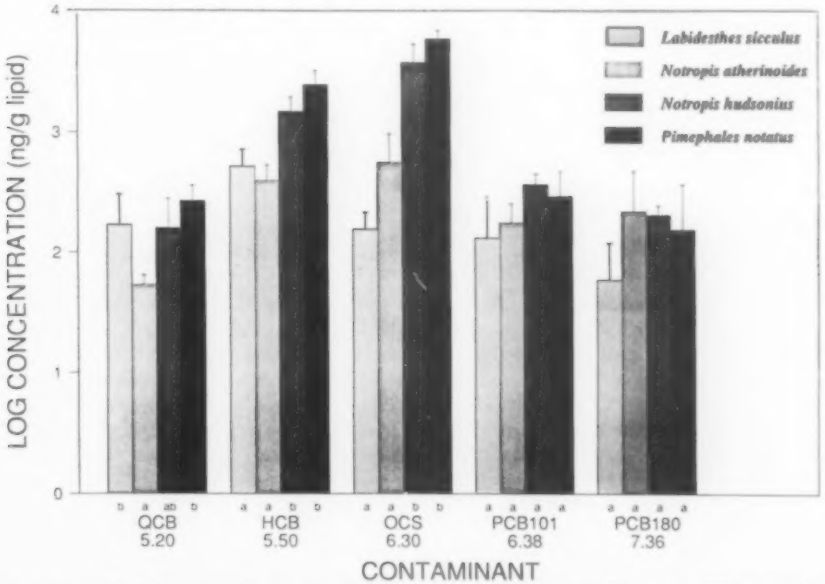


FIG. 2. Log_{10} lipid-normalized mean contaminant levels from the St. Clair River site, 1987. Error bars represent 1 SD. Bars with different letters are significantly different ($p < 0.05$).

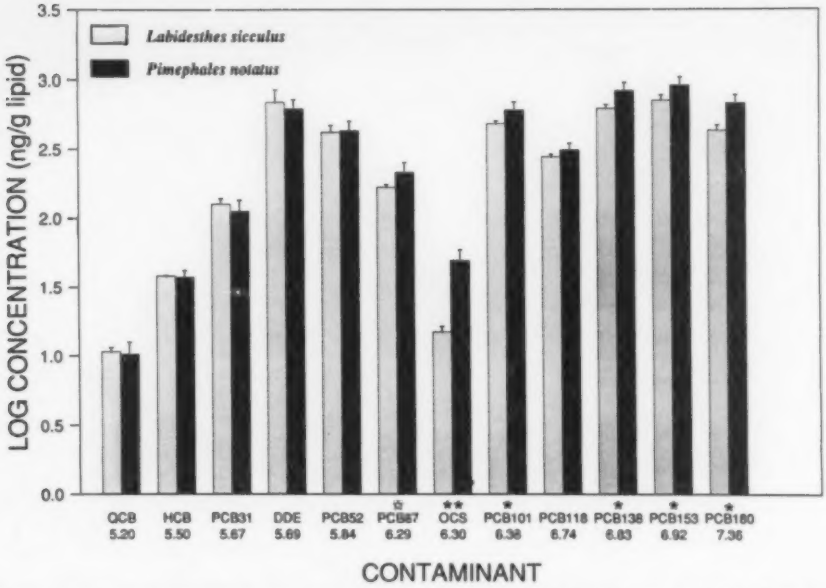


FIG. 3. Log_{10} lipid-normalized mean contaminant levels from the Detroit River site, 1988. Error bars represent 1 SD. Significance levels: $^{**}p < 0.01$; $^{*}p < 0.05$; open star, $p < 0.06$.

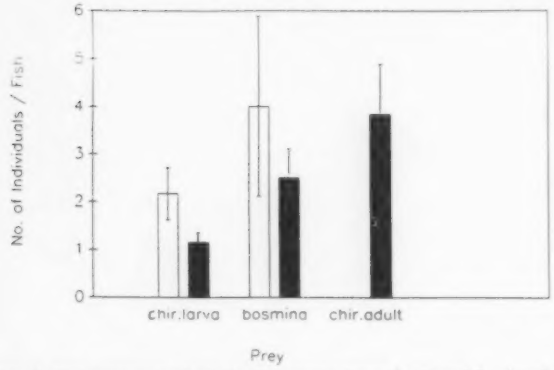


FIG. 4. Gut content analysis for *P. notatus* (open bars) and *L. sicculus* (solid bars). Error bars represent 1 SE.

nificant difference in the slopes or intercepts ($p > 0.1$) of the lines for the two species. However, this may be because of the relatively poor fit of log BAF to log K_{ow} on a linear relationship.

Discussion

Thermodynamic models would predict that there would be little or no interspecific differences in lipid-normalized contaminant burdens in fish. This study is among the first to present evidence of different contaminant levels in philopatric species of fish of the same age, size, lipid content, and trophic status. Earlier studies indicated that food can play an important role in determining contaminant levels (Thomann and Connolly 1984; Oliver and Niimi 1988). When sediment or sediment-associated organisms are an important component of the diet, exposure via food can be of particular importance (Schindler 1987). The role that sediments have in contaminant transfer has often been downplayed because of the assumption that chemicals associated with sediments are highly bound and therefore not biolog-

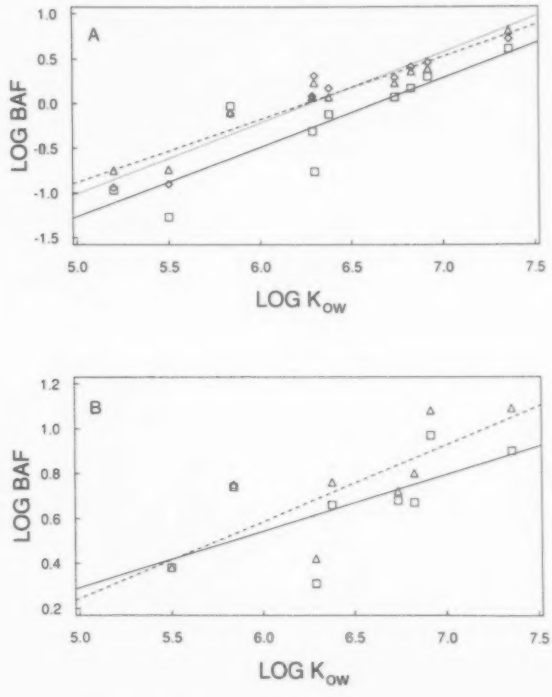


FIG. 5. Log bioaccumulation factor versus log octanol/water partition coefficient for (A) the St. Clair River site, 1988 (compounds examined, from left to right: QCB, HCB, PCB 52, 87, OCS 101, 118, 138, 153, 180; dotted line (diamonds) = *P. notatus*, broken line (triangles) = *N. hudsonius*, solid line (squares) = *L. sicculus*) and (B) the Detroit River site, 1988 (compounds examined, from left to right: HCB, PCB 52, 87, 101, 118, 138, 153, 180; broken line (triangles) = *P. notatus*, solid line (squares) = *L. sicculus*).

ically available (Neff 1984). In most ecosystems, however, many physical and biological processes may re-introduce these compounds into aquatic food chains (Reynoldson 1987; Riedel et al. 1987). Previous studies have shown the potential for contaminant uptake from sediments by invertebrates (Oliver 1984; Larsson 1986; Oliver 1987) and fish. Larsson (1986) concluded that sediment-mediated uptake by fishes could occur by (1) bioturbation, desorption, and gas convection allowing contaminants to move across the sediment–water interface and be bioconcentrated and (2) food chain transfer which initially involves bioaccumulation by benthic invertebrates followed by transfer to predatory fishes. Seelye et al. (1982) and Willford et al. (1987) indicated that contaminant transfer to fish need not be limited to a food chain mechanism but that accumulation can occur directly from the sediment by detritivores.

The interspecific differences in contaminant burdens we found indicate that food and habitat selection are major factors regulating contaminant levels in forage fish. In the St. Clair and Detroit rivers, interspecific differences were associated primarily with the higher K_{ow} compounds. There were no significant differences among levels of low-K_{ow} compounds, such as pentachlorobenzene, which would be relatively water soluble compared with the other compounds studied. In the Detroit River, no interspecific differences were seen between the surface and benthic feeders for any chemical with a log $K_{ow} < 6.0$ indicating that for these lower K_{ow} compounds, exposure through water may be the primary route of transfer. Exposure to chemicals through water would be more homogeneous than through food because low-K_{ow} compounds would be uniformly distributed in the aqueous phase whereas individual prey items may vary in their chemical concentrations. High-K_{ow} compounds with low aqueous solubility, such as OCS and PCB 180, tend to be associated more with the particulate phase than the water phase. When feeding, *P. notatus* comes into contact with the sediment and will ingest organic detritus. As a result of feeding on benthic organic detritus and zoobenthos, *P. notatus* had consistently higher levels of the higher K_{ow} contaminants. *Notropis hudsonius*, a facultative benthivore, also had significantly higher contaminant burdens than *L. sicculus* and *N. atherinoides* but lower levels than *P. notatus*. This difference might be due to this species' selection of a wider variety of food items and less dependence on organic detritus than *P. notatus*. The levels of contaminants in *L. sicculus* were much lower than in the two benthic species because a large proportion of its diet consists of terrestrial invertebrates. These is little or no direct contact between *L. sicculus* and the organic carbon pool of the sediments.

Species associated with the benthic community had higher bioaccumulation factors than the surface-feeding species. Because the slopes of the lines were similar the uptake and depuration mechanisms regulating bioaccumulation are similar among species. Thus, interspecific differences in metabolism of these compounds are negligible. Hebert (1990) has shown that the chemical elimination rate constants for QCB, HCB, and OCS are similar for *L. sicculus* and *P. notatus*. The different BAFs observed among species of forage fish reflect that exposure dynamics are species dependent.

In the St. Clair River, significant differences were observed between the intercepts of the lines for the benthivores versus the surface feeder. In the Detroit River, however, no significant differences between the intercepts of *P. notatus* and *L. sicculus* were observed. The latter was predicted for the lower K_{ow} compounds, as exposure to these less hydrophobic compounds should be more homogeneous, with water playing a more important role as a source of contamination (Haffner et al. 1991). The interspecific differences for low-K_{ow} compounds observed at the St. Clair River site may be a result of the nearby source of the contaminants. In the St. Clair River, inputs of the lower K_{ow} compounds, such as QCB and HCB, are from nearby point sources, and therefore, levels in the sediment are very high and have not equilibrated with the water column. In the St. Clair River, point sources of PCBs are not as important as in the Detroit River. Therefore, at the St. Clair River site the primary source of many of the higher K_{ow} compounds may have been through atmospheric deposition. In the Detroit River there is evidence of the expected pattern of low-K_{ow} compounds being more homogeneously distributed in the food web and considerable compartmentalization being observed for the higher K_{ow} compounds. Levels of QCB and HCB are much lower in the Detroit River than in the St. Clair River and PCB contamination is higher as a result of point-source contamination. Thus, the proximity of a source of a contaminant and the establishment of chemical equilibria between the sediments and water may have a role in regulating interspecific differences in these fish species.

It is apparent that resource partitioning can regulate the distribution of organic contaminants in aquatic ecosystems. This process is more important for higher K_{ow} compounds in that interspecific differences become more pronounced for compounds with a log $K_{ow} > 6.0$. Resource partitioning is an important process in the coupling of benthic and pelagic food webs and provides a mechanism to directly transfer contaminants from the sediment to valued game fish. The association of chemical levels with resource utilization supports the findings of Flint et al. (1988) that these chemicals can be used to quantify benthic and pelagic carbon flow in aquatic ecosystems. Consequently, chemical exposure dynamics in aquatic ecosystems are dependent on trophic organization as well as chemical properties such as K_{ow}.

The results of this study have important implications for biomonitoring programs (Suns et al. 1985) in that species-specific differences in chemical levels can occur in philopatric communities of cyprinids. There is no justification that a single species within a given trophic level can be considered an ecological equivalent.

References

BORGMANN, U., AND D. M. WHITTLE. 1983. Particle-size-conversion efficiency and contaminant concentrations in Lake Ontario biota. Can. J. Fish. Aquat. Sci. 40: 328–336.

CANADIAN WILDLIFE SERVICE. 1982. Analysis of organochlorine hydrocarbons in tissue samples. Analytical manual. Environment Canada, Ottawa, Ont.

CONNOLLY, J. P., AND C. J. PEDERSON. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environ. Sci. Technol. 22: 99–103.

FLINT, R. W., W. H. MCDOWELL, AND G. YOGIS. 1988. Potential use of organochlorine contaminants to validate a food web model. Verh. Int. Ver. Limnol. 23: 265–270.

GOBAS, F. A. P. C., D. C. G. MUIR, AND D. MACKAY. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17: 943–962.

HAFFNER, G. D., F. A. P. C. GOBAS, AND D. S. LAVIOLETTE. 1991. The distibution of organic contaminants in the Lake St. Clair food web: patterns and problems. Hydrobiologia. (In press)

HEBERT, C. E. 1990. Factors regulating organochlorine contaminant levels in forage fish from the Huron–Erie Corridor. M.Sc. thesis, University of Windsor, Windsor, Ont. 105 p.

JOHNSON, M. G., J. R. M. KELSO, AND S. E. GEORGE. 1988. Loading of organochlorine contaminants and trace elements to two Ontario lake sys-

tems and their concentrations in fish. Can. J. Fish. Aquat. Sci. 45: 170–178.

KAUSS, P. B., AND Y. S. HAMDY. 1985. Biological monitoring of organochlorine contaminants in the St. Clair and Detroit rivers using introduced clams, *Eliptio complanatus*. J. Great Lakes Res. 11: 247–263.

LARSSON, P. 1986. Zooplankton and fish accumulate chlorinated hydrocarbons from contaminated sediments. Can. J. Fish. Aquat. Sci. 43: 1463–1466.

MUTH, K. M., AND W. N. BUSCH. 1989. Food of forage fishes in western Lake Erie, 1975–76. J. Great Lakes Res. 15: 217–222.

NEELY, W. B., D. R. BRANSON, AND G. E. BLAU. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Technol. 8: 1113–1115.

NEFF, J. M. 1984. Bioaccumulation of organic micropollutants from sediments and suspended particulates by aquatic animals. Fresenius Z. Anal. Chem. 319: 132–136.

OLIVER, B. G. 1984. Uptake of chlorinated organics from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. 41: 878–883.

1987. Bio-uptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol. 21: 785–790.

OLIVER, B. G., AND A. J. NIIMI. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ. Sci. Technol. 22: 388–397.

REYNOLDSON, T. B. 1987. Interactions between sediment contaminants and benthic organisms. Hydrobiologia 149: 53–66.

RIEDEL, G. F., J. G. SANDERS, AND R. W. OSMAN. 1987. The effect of biological and physical disturbances on the transport of arsenic from contaminated estuarine sediments. Estuarine Coastal Shelf Sci. 25: 693–706.

SCHINDLER, D. W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 6–25.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184.

SEELYE, J. G., R. J. HESSELBERG, AND M. J. MAC. 1982. Accumulation by fish of contaminants released from dredged sediments. Environ. Sci. Technol. 16: 459–463.

SUNS, K., G. CRAWFORD, AND D. RUSSELL. 1985. Organochlorine and mercury residues in young-of-the-year spottail shiners from the Detroit River, Lake St. Clair, and Lake Erie. J. Great Lakes Res. 11: 347–352.

THOMANN, R., AND J. CONNOLLY. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18: 65–71.

VEITH, G. D., D. I. DEFOE, AND B. V. BERGSTEDT. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36: 1040–1048.

WILLFORD, W. A., M. J. MAC, AND R. J. HESSELBERG. 1987. Assessing the bioaccumulation of contaminants from sediments by fish and other aquatic organisms. Hydrobiologia 149: 107–111.

Fecundity and Reproductive Output in Nine Species of *Cancer* crabs (Crustacea, Brachyura, Cancridae)[1]

Anson H. Hines

Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037, USA

Hines, A. H. 1991. Fecundity and reproductive output in nine species of Cancer crabs (Crustacea, Brachyura, Cancridae). Can. J. Fish. Aquat. Sci. 48: 267–275.

The major variables of reproductive output and fecundity were compared among brooding females of nine species of *Cancer* from the North Pacific and North Atlantic: *C. oregonensis*, *C. gracilis*, *C. irroratus*, *C. antennarius*, *C. productus*, *C. borealis*, *C. anthonyi*, *C. magister*, and *C. pagurus*. Female body size is the principal determinant of reproductive output, with dry body weights spanning two orders of magnitude from 1.2 to 199.5 g among species and often one order of magnitude within species. Mean dry brood weights ranged from 0.21 to 26.7 g among species, with relative brood size varying from 11 to 19% of female weight. The brood mass is partitioned into eggs ranging among species from 311 to 442 μm, resulting in mean fecundities ranging among species from 18 200 to 2 208 000 eggs per brood. Most species produce one or two broods per year over the winter–spring season over a reproductive span averaging 6.6 yr (range 4–10 yr). Cumulative reproductive output and cumulative fecundity over the maximum estimated life span exhibited approximately isometric functions of maximum body size, and they ranged among species from 1.88 to 316 g brood weight and 158 000 to 22 300 000 eggs per lifetime, respectively. The pattern of covariation of reproductive traits in the Cancridae corresponds well with the overall pattern previously determined for a diverse array of brachyurans from seven families.

Les paramètres principaux de la production d'oeufs et de la fécondité ont été comparés chez les femelles oeuvées de neuf espèces de *Cancer* du Pacifique Nord et de l'Atlantique Nord : *C. oregonensis*, *C. gracilis*, *C. irrotatus*, *C. antennarius*, *C. productus*, *C. borealis*, *C. anthonyi*, *C. magister* et *C. pagurus*. La taille des femelles est le facteur le plus fortement corrélé à la production d'oeufs; le poids corporel sec mesuré variait de deux ordres de grandeur dans l'ensemble des espèces, allant de 1,2 à 199,5 g, et différait souvent d'un ordre de grandeur entre les individus d'une même espèce. Le poids moyen de la ponte variait de 0,21 à 26,7 g pour l'ensemble des espèces, et le poids de la ponte d'une femelle représentait entre 11 et 19 % de son poids. Pour l'ensemble des espèces, la taille des oeufs variait de 311 à 442 μm et le nombre d'oeufs par ponte (fécondité), de 18 200 à 2 208 000. La plupart des espèces produisent une ou deux pontes par année durant l'hiver et le printemps sur une période de 6,6 ans en moyenne (4 à 10 ans). La production pondérale d'oeufs et la fécondité cumulatives pour la durée de vie maximale estimée montrent une relation approximativement isométrique avec la taille corporelle maximale et varient parmi les espèces de 1,88 à 316 g (poids total des oeufs) et de 158 000 à 22 300 000 oeufs, respectivement, pour toute la durée de la vie des femelles. Les rapports de covariance des paramètres caractérisant la reproduction chez les Cancridae correspondent avec les relations générales établies antérieurement chez divers brachyoures de sept familles.

Received October 31, 1989
Accepted August 10, 1990
(JA349)

Reçu le 31 octobre 1989
Accepté le 10 août 1990

The principal determinant of fecundity per brood and reproductive output (brood mass) in female brachyuran crabs is body size, such that brood weight is generally constrained to about 10% of female body weight by the space available for yolk accumulation in the cephalothorax (Hines 1982). Egg size is the major reproductive trait which is highly variable among brachyuran species (Hines 1982, 1986a). This high interspecific variability in how the relatively constrained brood mass is partitioned (many small or few large eggs) results in much greater interspecific variation in fecundity than in brood mass for equivalent-sized crabs (Hines 1982). Because of the relative constraint on brood mass, the number of broods produced per year is probably the other key reproductive trait which exhibits high interspecific variability in brachyuran life history strategies (Hines 1982). In combination with these size-specific reproductive variables, age and size of mature instars determine the schedules of reproductive output and fecundity (the m_x schedule of life tables; Deevey 1947) throughout the reproductive life of a brachyuran species.

The ecological and evolutionary consequences of these apparent covariations of reproductive traits have not been studied extensively in any group of closely related brachyuran species in similar habitats (Hines 1986a). However, I recently compared their covariation in two species of large deep-sea crabs in the family Geryonidae (Hines 1988). As in other crab species, body size was the primary determinant of reproductive output in the deep-sea species; however, their brood masses at 16 and 22% of body weight are considerably larger than the 10% average for other crab species and are near the upper extreme of apparently typical interspecific variation. They also had very large eggs and relatively low fecundity per brood compared with many species of similar size. Comparison of covariation in reproductive traits between the two species showed that one (*Geryon quinquedens*) had a 50% larger volume of the body

[1]Contribution No. 260 of the Smithsonian Marine Station.

cavity, resulting in a 50% larger brood mass, than the other (*G. fenneri*). Despite its larger brood mass, egg size in *G. quinquedens* was about twice as large as in *G. fenneri*, resulting in about equal size-specific fecundity per brood in the two species. There are no other published comparisons of reproductive traits among an array of closely related brachyuran species which span a broad size range.

The brachyuran genus *Cancer* comprises 23 extant species distributed throughout the cold temperate and boreal zones of the Pacific and Atlantic oceans (Mackay 1943; Nations 1975, 1979). Species in the genus span a wide range of body size at maturity. Because several of the larger species are conspicuous and form the basis of historically lucrative or of currently developing fisheries, the biology of several important commercial species is well studied: *C. magister* (e.g. Poole 1966; Wild and Tasto 1983; Melteff 1985), *C. pagurus* (e.g. Hancock and Edwards 1967; Ingle 1981), *C. anthonyi* (Anderson and Ford 1976; Shields 1987), *C. antennarius* (Carroll 1982), *C. productus* (Trask 1970), and *C. borealis* (Haefner 1977; Sastry 1977; Carpenter 1978). Although not commercially important, *C. irroratus* is also relatively well studied (e.g. Krouse 1976; Haefner 1976; Reilly and Saila 1978) because of its ecological importance in shallow subtidal communities of the North Atlantic and also apparently because of its proximity to concentrations of marine biologists in northeastern North America. The biology of the nine species which occur along the west coast of North America has been reviewed (Garth and Abbott 1980), and Orensanz and Gallucci (1988) recently provided valuable new information on the growth and life histories of *C. oregonensis*, *C. gracilis*, and *C. productus*. Thus, data on the rate of brood production, as well as size and age of reproductive instars, can be extracted from the literature for at least eight species of *Cancer*. However, there has been no detailed analysis of the group's reproductive patterns employing appropriate allometric, statistical, and demographic methods for interspecific comparisons. Moreover, most of these key reproductive variables have been inadequately quantified, even though they comprise demographic traits which should be considered for sound fisheries management of commercially important species.

This study compares brood weight, fecundity per brood, and egg size as a function of female body size in nine species of *Cancer* from the north-temperate and boreal and zones of the eastern Pacific and Atlantic oceans. In combination with data on age, size, and brood production rates extracted from the literature, these size-specific reproductive variables are used to estimate the schedules of brood production and fecundity over the maximum life span of eight of the species. I also compare the pattern of covariation in reproductive traits in the genus with the apparently general pattern for brachyurans determined from species representing a diverse array of families (Hines 1982).

Methods

Samples of 5–15 ovigerous females of each of nine species of *Cancer* were obtained from locations and sources listed in Table 1. The samples selected females which spanned the size range of ovigerous females collected from populations at the sites. Although the size range of sampled crabs did not necessarily span the range reported in the literature for reproductive females, the range of sampled crabs was in all cases more than adequate to determine the size-dependent regressions for reproductive variables (see Results). The collections selected crabs with brooded embryos in developmental stages from late blastula to early gastrula to avoid confounding effects of embryonic diameters swelling during late stages of development or of egg loss from the brood during incubation. The samples were frozen and then fixed in 10% formalin–seawater and stored in 70% ethanol until they were processed. Processing followed the method of Hines (1982, 1988), in which the following variables were measured for each crab: maximum (spine-to-spine) carapace width (millimetres) of the female, female dry body weight (grams), dry weight (grams) of the brooded egg mass, average volume (cubic millimetres) of an egg, calculated for a sphere

TABLE 1. Collecting information for nine species of *Cancer*.

Species	No. of females	Collecting site	Depth (m)	Collecting method	Date
C. oregonensis	14	Puget Sound, Washington	0–10	Hand, dredges	April 1985
C. gracilis	12	Puget Sound, Washington	36	Trawl, dredges	April 1985
C. irroratus	10	Boothbay Harbor, Maine	3–10	Lobster trap	July 1984
C. antennarius	10	Humboldt Bay, California	30	Crab pot	December 1980
C. productus	6	Humboldt Bay, California	30	Crab pot	February 1984
C. borealis	11	Boothbay Harbor, Maine	3–10	Lobster trap	July 1984
C. anthonyi	8	Santa Barbara, California	15–70	Crab pot	September 1982
C. magister	10	Gulf of Farallones, California	35–75	Crab pot	December 1980
C. pagurus	5	Scotland	12–45	Crab pot, dredge	December 1985, March 1986

TABLE 2. Size and reproductive regressions for nine species of *Cancer* (ns = not significant).

	log body wt. = b(log carapace width) + c	R^2	log brood wt. = b(log body wt.) + c	R^2	log no. of eggs = b(log body wt.) + c	R^2
C. oregonensis	$y = 3.041x - 4.089$	0.995	$y = 1.075x - 0.757$	0.978	$y = 1.026x + 4.188$	0.965
C. gracilis	$y = 2.896x - 4.134$	0.904	$y = 1.049x - 0.851$	0.812	$y = 1.081x + 4.392$	0.946
C. irroratus	$y = 2.612x - 3.656$	0.736	$y = 1.393x - 1.472$	0.725	$y = 1.187x + 3.781$	0.703
C. antennarius	$y = 2.745x - 3.663$	0.945	$y = 1.349x - 1.530$	0.922	$y = 1.518x + 3.285$	0.929
C. productus	$y = 2.523x - 3.379$	0.983	$y = 0.838x - 0.429$	0.965	$y = 0.711x + 4.462$	0.854
C. borealis	$y = 2.547x - 3.443$	0.722	$y = 1.282x - 1.476$	0.698	$y = 1.137x + 3.711$	0.621
C. anthonyi	$y = 2.896x - 4.093$	0.973	$y = 1.011x - 0.855$	0.948	$y = 0.995x + 4.354$	0.960
C. magister	$y = 0.378x + 1.292$	0.004 ns	$y = 0.527x + 0.229$	0.644	$y = 0.611x + 4.677$	0.629
C. pagurus	$y = 4.119x - 6.813$	0.959	$y = 1.246x - 1.440$	0.956	$y = 1.152x + 3.499$	0.955

TABLE 3. Means and ranges of body size and reproductive variables for nine species of *Cancer*. "Observed min.–max." indicates range of observations for a variable in the sample for each species. "Predicted min.–max." indicates range predicted by the regressions in Table 2 for the smallest and largest individuals sampled for each species.

	Body size		Egg size	Brood size		Fecundity
	$\bar{x}$ carapace width (mm) (observed min.–max.)	$\bar{x}$ dry wt. (g) (observed min.–max.)	$\bar{x}$ diameter (μm) (observed min.–max.)	$\bar{x}$ dry wt. (g) (observed min.–max.) (predicted min.–max.)	$\bar{x}$ % of body wt.	$\bar{x}$ no. of eggs/brood (observed min.–max.) (predicted min.–max.)
C. oregonensis	23 (8–27)	1.2 (0.05–5.1)	383 (296–489)	0.21 (0.007–0.085) (0.007–1.008)	17.5	18 200 (780 to 82 500) (713 to 82 000)
C. gracilis	68 (52–81)	14.7 (6.7–23)	329 (294–352)	2.36 (0.3–5.5) (1.0–3.8)	16.1	453 700 (189 300 to 789 400) (192 700 to 731 200)
C. irroratus	78 (66–89)	19.5 (13–31)	406 (332–441)	2.11 (1.2–4.3) (1.2–4.0)	10.8	205 200 (101 600 to 356 400) (126 800 to 355 800)
C. antennarius	100 (65–120)	67.6 (20–118)	333 (306–374)	8.68 (1.6–24.0) (1.7–18.4)	12.8	1 156 000 (198 500 to 3 004 000) (182 000 to 2 692 000)
C. productus	116 (92–142)	67.6 (39–107)	367 (340–401)	12.7 (7.6–18.9) (8.0–18.7)	18.8	877 300 (559 000 to 1 036 000) (593 300 to 1 216 000)
C. borealis	120 (105–135)	70.8 (46–95)	428 (353–470)	7.87 (3.7–12.4) (4.5–11.5)	11.1	652 300 (311 400 to 1 045 000) (399 500 to 1 802 000)
C. anthonyi	127 (86–153)	100.0 (33–173)	311 (278–331)	14.7 (4.1–23.5) (4.8–25.6)	14.7	2 208 000 (680 100 to 3 849 000) (732 700 to 3 809 000)
C. magister	155 (145–170)	131.8 (89–251)	442 (412–462)	22.2 (15.5–32.6) (18.0–31.1)	16.8	938 300 (658 600 to 1 342 000) (738 000 to 1 391 000)
C. pagurus	163 (140–184)	199.5 (108–332)	396 (383–414)	26.7 (10.9–45.5) (12.4–50.3)	13.4	1 408 000 (605 600 to 2 310 000) (694 200 to 2 531 000)

with the mean measured diameter (micrometres) including the chorionic membrane tightly adhering to the embryonic surface of a subsample of seven eggs before drying, and the number of eggs per brood, extrapolated from the dry weights of the total brood and a counted subsample of about 2000 eggs. At the time of egg measuring and counting, all broods were examined under a dissecting microscope for the presence of nemertean worms or other potential infections by egg predators.

Published data on the number, age, size, and brood production rates of mature instars were extracted from the literature for females of eight of the nine species sampled. These data were combined with the size-specific reproductive output determined empirically from the sampled crabs to estimate schedules of brood mass production and fecundity over the maximum life span of the species and to estimate the cumulative maximum lifetime brood weight and cumulative maximum lifetime fecundity for the species.

Data were analyzed with regressions of log–log transformations for allometric plots ($y = cX^b$; $\log y = b \log X + c$) of the reproductive variables versus dry weight. Comparisons

of different-sized crabs utilized ANCOVA with female body weight as the covariate after testing for homogeneity of variances with F_{max} tests (Sokal and Rohlf 1981) and equality of slopes, as discussed in Hines (1982).

Results

Mature females sampled within most of the nine species varied widely in body size. The sample of *C. oregonensis* had the largest range in size, with the largest individual being 4.6 times the carapace width and 102 times the dry body weight of the smallest individual (Tables 2 and 3). In contrast, the sample of *C. magister* ranged only 1.2-fold in carapace width and that of *C. borealis* ranged only 2.1-fold in dry body weight from the smallest to largest individuals (Tables 2 and 3). These size ranges were judged to be adequate samples of variation within species for three main reasons: (1) analyses of size dependence in reproductive variables yielded significant regression coefficients in all nine species, which is an important component of the subsequent allometric considerations (Table 2); (2) with careful consideration of exceptions, the ranges of most of the samples closely matched the size range of reproductive females reported in the literature (see below, Tables 3 and 4); and (3) the mean sizes of sampled females closely matched the average size of reproductive females reported in the literature for each of the nine species (see below, Tables 3 and 4). Mean body size of the females among the species ranged over sixfold in carapace width and about two orders of magnitude in dry body weight from 23 mm and 1.2 g, respectively, in *C. oregonensis* to 163 mm and 200 g, respectively, in *C. pagurus* (Tables 2 and 3; Fig. 1). Log body weight differed significantly among species (ANOVA, $F_{(8,81)} = 66.04$, $P < 0.001$). However, all of the species except the smallest (*C. oregonensis*) overlapped considerably in body size, and even *C. oregonensis* represented a clear continuum in body size of the group (Table 3; Fig. 2 and 3).

Nemertean worms (*Carcinonemertes* spp.) were observed in the egg masses of only one species (*C. magister*), but these infections were in their earliest stages and judged not to have impacted the egg masses significantly (Wickham 1979a, 1979b, 1980, pers. comm.).

Mean egg size ranged from 311 μm in diameter and 0.0157 mm^3 in volume for *C. anthonyi* to 442 μm and 0.0452 mm^3 for *C. magister* (Table 2; Fig. 1). Egg size did not vary significantly within species (ANOVA, $P > 0.05$). Mean egg size also was not correlated with female body size among species (Fig. 1; Table 2; ANCOVA, $F_{(8,81)} = 0.40$, $P > 0.05$).

Brood weight increased significantly with female body weight for all species (Fig. 2; Table 2; ANCOVA, $P < 0.05$); however, regression slopes did not differ significantly among species (ANCOVA, $F_{(8,81)} = 1.28$, $P > 0.2$). As a result of the size differences among species, brood size differed significantly among species, with mean dry brood weights ranging from only 0.21 g in *C. oregonensis* to over 26 g in *C. pagurus* (Fig. 1; Table 3; ANOVA on log brood weight, $F_{(8,81)} = 94.32$, $P < 0.001$). When adjusted for differences in female body size, least squares mean brood weights were similiar but still differed significantly and ranged from about 2.6 g in *C. pagurus* to 10.28 g in *C. magister* (Fig. 1; ANCOVA on log brood weight with log body weight as a covariate, $F_{(8,81)} = 2.17$, $P < 0.05$). Brood weight as a proportion of female

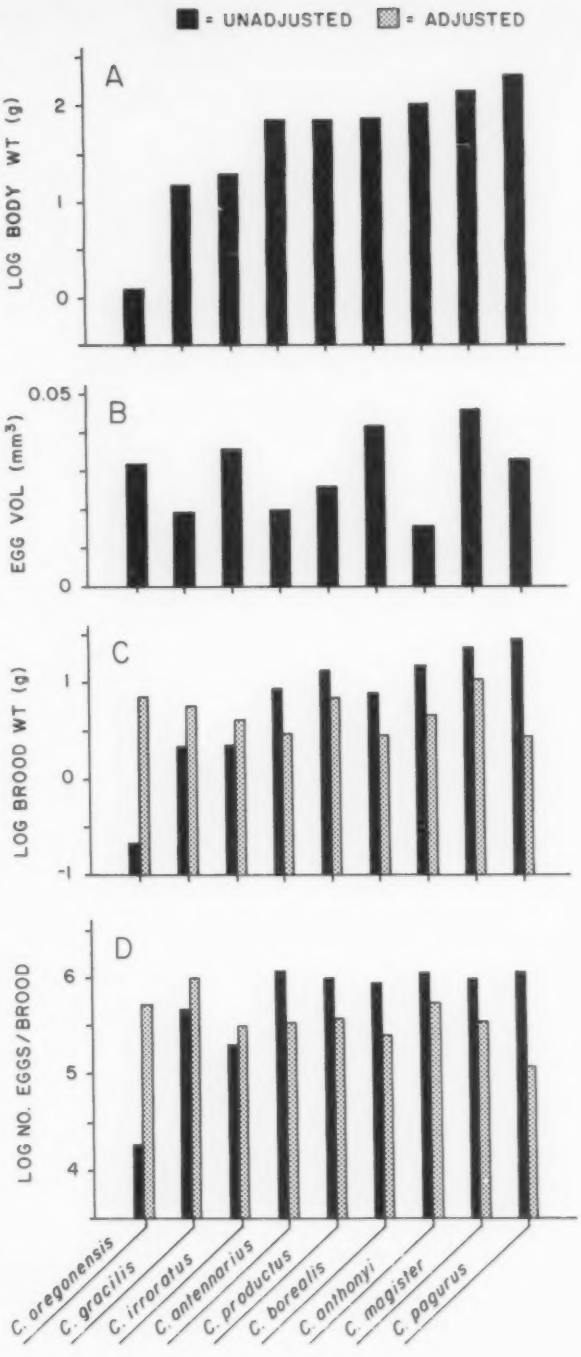


FIG. 1. Comparison of body size and reproductive variables for nine species of *Cancer* listed in Table 1. (A) Dry body weight; (B) egg volume; (C) dry brood weight; (D) number of eggs per brood. Plotted values are either arithmetic means (unadjusted for variation in body size) or least squares means (adjusted for variation in body size) from ANCOVA with log body weight as the covariate. Note log scale of Fig. 1A, 1C, and 1D.

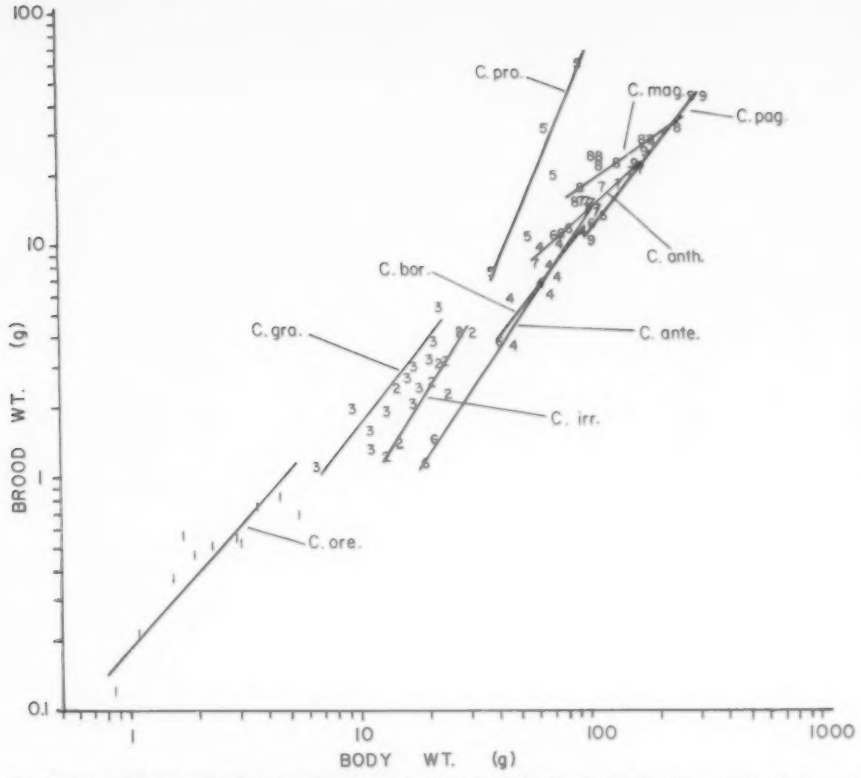


FIG. 2. Comparison of dry brood weight as a function of dry body weight for nine species of *Cancer* listed in Table 1. Regression equations are given in Table 2. Numbers coded for each species plot the points for each brood. Note log scales.

body weight ranged from 10.8% in *C. irroratus* to 18.8% in *C. productus*.

Fecundity per brood increased significantly with female body size in all nine species (Fig. 3; Table 2; ANOVA, $F = 197.98$, $P < 0.001$), and only *C. magister* differed significantly (lower) in regression slope for log egg number versus log body weight (Table 2; ANCOVA, $P < 0.05$). As a result of the differences in female body size, fecundities per brood differed significantly among species, with mean fecundities ranging from only about 18 000 eggs per brood in *C. oregonensis* to over 2 200 000 eggs in *C. anthonyi* (Fig. 1 and 3; Table 3; ANOVA, $F_{(8,81)} = 133.45$, $P < 0.001$). When adjusted for differences in female body size, least squares mean log numbers of eggs per brood were similar but still differed significantly among species and ranged from about 158 000 eggs per brood in *C. pagurus* to about 977 000 eggs in *C. gracilis* (Fig. 1; ANCOVA on log number of eggs with log body weight as a covariate, $F_{(8,81)} = 2.39$, $P < 0.05$).

Estimates of the instar number, age, and size of mature females extracted from the literature corresponded well with the size range of ovigerous crabs in my samples of the nine species, although the minimum and maximum sizes reported for some species exceeded the sampled range in a few species (Table 4). No data are available for growth in *C. borealis*, so data on instar number, size, and age could not be extracted from the literature, and cumulative maximum lifetime brood production and fecundity could not be estimated for this species. Most species produce a single brood each winter, although up to two or three broods per year have been reported for *C. gracilis*, *C. antennarius*, *C. productus*, and *C. anthonyi* (Table 4). Females of most other species mated and molted to a new instar each year, although larger individuals of the largest species (especially *C. magister* and *C. pagurus*) and perhaps *C. irroratus* are reported to skip molting in some years (e.g. Butler 1961; Hancock and Edwards 1967; Bennett 1974; Haefner and Van Engel 1975; Hankin et al. 1985). Age at maturity ranged from as young as 0.5 yr in *C. oregonensis* to as old as 4 yr in *C. pagurus*, although most species apparently mature at 1–2 yr old. The reproductive life span in the group ranged from about 4 yr in *C. gracilis*, *C. productus*, and *C. magister* to about 7 yr or more in *C. antennarius* and *C. pagurus*, with an average of 5 yr for the group (Table 4). The cumulative number of broods produced over the maximum life span of each species ranged from about 4 broods in *C. productus* and *C. magister* to about 10 in *C. antennarius* and *C. anthonyi*, with an average of 6.6 for the group. Although the smallest species (*C. oregonensis*) had the earliest age at maturation, neither the reproductive life span nor the cumulative number of broods produced was dependent on body size. Cumulative maximum lifetime brood weight (Table 4) ranged from 1.88 g in *C. oregonensis* to 316 g in *C. pagurus*; and it is a linear, isometric function of maximum body size, with very little variation among the species (Fig. 4A; log Cumbroodwt = 1.078 log Maxbodwt − 0.457; ANOVA, $F_{(1,6)} = 498.73$, $P < 0.0001$, $R^2 = 0.99$). Cumulative maximum lifetime fecundity (Table 4) ranged from 158 000 eggs in *C. oregonensis* to about 22 300 000 in *C. anthonyi*. Cumu-

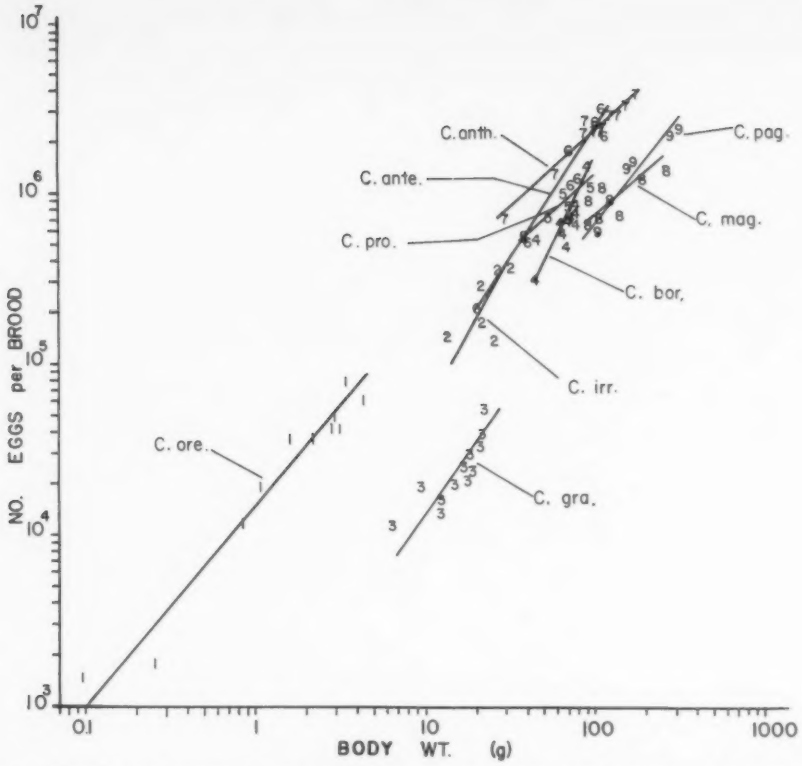


FIG. 3. Comparison of number of eggs per brood as a function of dry body weight for nine species of *Cancer* listed in Table 1. Regression equations are given in Table 2. Numbers coded for each species plot the points for each brood. Note log scales.

lative fecundity also is an approximately linear, isometric function of maximum body size, but variation among species about the regression is considerably greater than for brood weight (Fig. 4B; log Cumfecund = 1.003 log Maxbodwt + 4.621; ANOVA, $F_{(1,6)} = 49.82$, $P < 0.001$, $R^2 = 0.89$).

Discussion

The basic patterns of reproductive variables and constraints in the Cancridae conform generally well with those determined earlier for a broad array of brachyuran species (Hines 1982). Female body size is clearly the principal determinant of reproductive output and fecundity in the nine cancrid species, which spanned two orders of magnitude in mean body weight among species and often ranged an order of magnitude in body weight among ovigerous individuals within species. As a result of variation in body size, reproductive output and fecundity per brood also ranged about two orders of magnitude among species and one order of magnitude among individuals within species. Although the species differed significantly in brood weight and fecundity per brood after adjusting statistically for variation in body size, variation among species is relatively small. It is evident that brood size and fecundity are similar among equivalent-sized females (Fig. 1), with all species exhibiting a similar allometric relationship (Fig. 2 and 3). These cancrid species apparently produce a small number of broods per year, typically only about one or two over the winter season. Cumulative reproductive output and fecundity over the predicted life spans of each species exhibit nearly isometric relationships for the group, although cumulative fecundity is more variable than cumulative brood weight (Fig. 4). In contrast with reproductive output and fecundity, egg size did not vary with female size within or among species, but egg volume varied about threefold among species (Fig. 1). Thus, female body size and egg size appear to be the major variables of reproduction in the Cancridae, while brood weight adjusted for body size and the number of broods per season are relatively constant. Fecundity per brood and fecundity per life span appear to follow as direct consequences of female size limits on reproductive output, albeit with increased variance.

The comparative data reported for reproductive variables of the nine cancrids in this paper are generally corroborated by the literature on individual species. For some species, the size range of reproductive females is wider than those of my relatively limited samples, especially for *C. borealis*, *C. antennarius*, *C. productus*, *C. magister*, and *C. pagurus* (compare sizes in Tables 3 and 4). Both maximum and minimum size at maturity vary geographically within some species, especially *C. irroratus* (Scaratt and Lowe 1972; Krouse 1976; Haefner 1976; Reilly and Saila 1978; Bigford 1979; Campbell and Eagle 1983). However, the size-dependent regressions provided from my samples allow comparative estimates of reproductive variables for all sizes of females within each species. There are essentially no other published measures for brood weight in cancrids, except by Hankin et al. (1985) for *C. magister* with somewhat smaller brood weights for equivalent-sized females (about 16–26 g versus about 16–32 g in my samples). Fecundities per brood in species with published estimates correspond

TABLE 4. Schedules of brood production and fecundity for nine species of *Cancer*. Data for instar, age, carapace width, and no. of broods are extracted from references. Dry brood wt., cumulative brood wt., no. of eggs per brood, and cumulative fecundity are derived from regressions in Table 2. "Cumulative brood wt." and "cumulative fecundity" are cumulative schedules for $\bar{x}$ no. of broods times the brood wt. or no. of eggs per brood for each age interval.

Species (references)	Instar (no.)	Age (yr)	Carapace width (mm)	Dry body wt. (g)	No. of broods $\bar{x}$	No. of broods (min.–max.)	Brood wt. (g)	Cumulative brood wt. (g)	No. of eggs per brood	Cumulative fecundity
C. oregonensis	5	0.5	12	0.156	1	(1–2)	0.024	0.024	2 290	2 290
(Orensanz and	6	1	16	0.374	1	(1–2)	0.061	0.085	5 620	7 910
Gallucci 1988)	7	2	21	0.855	1	(1–2)	0.148	0.233	13 200	21 100
	8	3	27	1.84	1	(1–2)	0.337	0.570	28 800	49 900
	9	4	31	2.79	1	(1–2)	0.527	1.10	43 700	93 600
	10	5	35	4.04	1	(1–2)	0.785	1.88	64 600	158 200
C. gracilis	9	1.5	47	5.11	1	(1)	0.780	0.780	143 800	143 800
(Orensanz and	10	2	62	11.4	1.5	(1–2)	1.81	2.59	342 400	486 200
Gallucci 1988)	11	3	77	21.3	1.5	(1–2)	3.49	6.08	672 900	1 159 100
	12	4	86	29.4	1	(1–2)	4.89	11.0	953 400	2 112 500
C. irroratus	9	3	51	6.37	1	(1)	0.445	0.445	54 400	54 400
(Reilly and Saila	10	4	61	10.2	1	(1)	0.857	1.30	95 100	149 500
1978; Haefner and	11	5	71	15.1	1	(1)	1.48	2.78	151 500	301 000
Van Engel 1975;	12	6	80	20.6	1	(1)	2.28	4.98	219 100	520 100
Krouse 1976)	13	7	89	27.3	1	(1)	3.38	8.36	306 000	826 100
	14	8	97	34.2	1	(1)	4.62	13.0	399 800	1 225 900
C. antennarius	10	1.5	62	18.1	1	(1)	1.47	1.47	156 400	156 400
(Carroll 1982)	11	2	71	26.2	1.5	(1–2)	2.42	3.89	274 100	430 500
	12	3	83	40.3	1.5	(1–2)	4.32	8.21	527 100	957 600
	13	4	97	61.8	1.5	(1–2)	7.69	15.9	988 900	1 946 600
	14	5	112	91.6	1.5	(1–2)	13.1	29.0	1 833 000	3 779 500
	15	6	128	132.0	1.5	(1–2)	21.4	50.4	3 192 000	6 971 500
	16	7	145	186.0	1.5	(1–2)	34.0	84.4	5 372 000	12 343 500
C. productus	10	1	59	12.3	1	(1–2)	3.05	3.05	172 600	172 600
(Orensanz and	11	2	78	24.8	1	(1–2)	5.49	8.54	284 100	456 700
Gallucci 1988)	12	3	107	55.1	1	(1–2)	10.7	19.2	501 100	957 800
	13	4	118	70.5	1	(1–2)	13.2	32.4	597 100	1 554 900
C. borealis	?	?	90	34.2	1	(1)	3.09	3.09	285 200	285 200
(Haefner 1977;	?	?	?	?	1	(1)	?	?	?	?
Carpenter 1978)	?	?	140	106.0	1	(1)	13.2	?	1 032 000	?
C. anthonyi	12	1	90	36.9	2	(1–3)	5.36	10.7	818 800	1 638 000
(Anderson and Ford	13	2	105	57.6	2	(1–3)	8.41	27.5	1 275 000	4 188 000
1976; Shields	14	3	120	84.8	2	(1–3)	12.4	52.3	1 569 000	7 326 000
1987)	15	4	150	130.0	2	(1–3)	19.2	90.7	2 359 000	12 044 000
	16	5	170	233.0	2	(1–3)	34.5	159.7	5 123 000	22 290 000
C. magister	10	2	108	115.0	1	(1)	20.7	20.7	886 200	886 200
Mackay and Weymouth	11	3	131	124.0	1	(1)	21.5	42.2	903 800	1 790 000
1935; Cleaver 1949;	12	4	161	134.0	1	(1)	22.4	64.6	947 800	2 737 800
Butler 1961;	13	5	182	140.0	1	(1)	22.9	87.5	973 400	3 711 200
Collier 1983;										
Hankin et al. 1985)										
C. pagurus	14	4	118	52.6	1	(1)	5.06	5.06	303 100	303 100
(Mackay 1942;	15	5	146	126.0	1	(1)	15.0	20.1	829 100	1 132 200
Edwards 1979;		6			1	(1)		35.1		1 961 300
Hancock and Edwards	16	7	177	280.0	1	(1)	40.7	75.8	2 080 000	4 041 300
1967; Bennett 1974)		8			1	(1)		116.0		6 121 300
	17	9	211	576.0	1	(1)	99.9	216.0	4 775 000	10 896 300
		10			1	(1)		316.0		15 671 300

closely with my samples. For example, numbers of eggs per brood in my samples for *C. magister* are virtually identical to estimates by Hankin et al. (1985) for equivalent-sized crabs. My size-dependent estimates of fecundity per brood are also similar to fecundity ranges reported by others for *C. oregonensis* (Knudsen 1964; Orensanz and Gallucci 1988), *C. gracilis* (Orensanz and Gallucci 1988), *C. irroratus* (Reilly and Saila 1978; Campbell and Eagle 1983), *C. antennarius* (Carroll 1982), *C. borealis* (Haefner 1977), *C. anthonyi* (Shields 1987; Shields 1991), and *C. pagurus* (Edwards 1979). *Cancer setosus*, another cancrid in the same size range as *C. magister*, produces 600 000 to 1 700 000 eggs per brood in females 83–

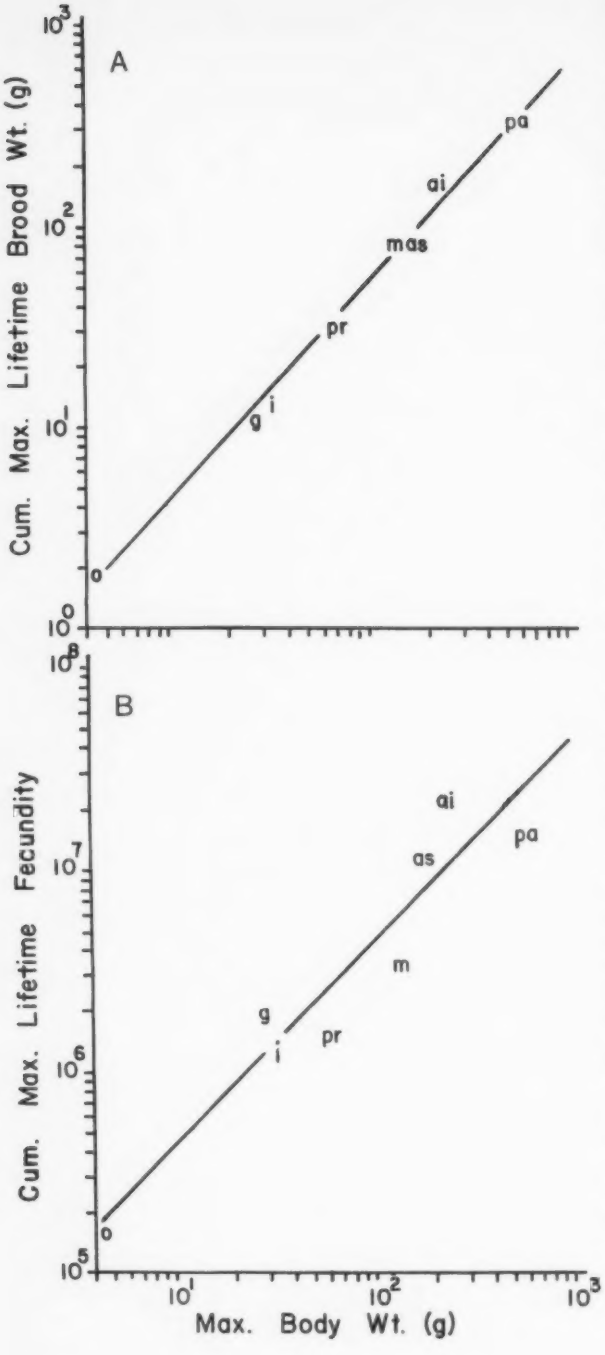


FIG. 4. Comparison of (A) cumulative brood weight and (B) cumulative fecundity over the maximum predicted life span of eight species of *Cancer*, as calculated in Table 4. Regression equations are given in text. Note log scales. Species codes: ai = *C. anthonyi*; as = *C. antennarius*; g = *C. gracilis*; i = *C. irroratus*; m = *C. magister*; o = *C. oregonensis*; pa = *C. pagurus*; pr = *C. productus*.

151 mm carapace width, respectively (Gutierez and Zuniga 1976), which is also about the fecundity of *C. magister*. The occurrence of "skip molting" in some larger females of some species may reduce fecundities per brood, perhaps because females lack adequate stored sperm without copulation between each brood, as suggested by Hankin et al. (1985) for *C. magister*. Extrapolated numbers of eggs per brood for the largest instars of *C. antennarius*, *C. anthonyi*, and *C. pagurus* (Table 4) are about 1.5 times higher than values directly estimated for any sampled crab. However, rigorous quantitative tests of this hypothesis are lacking in most species.

The pattern of covariation in reproductive traits in the Cancridae contrasts with that in the Geryonidae (Hines 1988). Relative brood size was somewhat smaller in the cancrids (11–19% of female body weight) than in the geryonids (16–22%). However, egg size in the cancrids is much smaller than in the geryonids (by nearly an order of magnitude in volume, about 0.03 mm^3 in cancrids versus about 0.15 mm^3 in geryonids), which have among the largest eggs of the Brachyura with planktotrophic larvae (Hines 1986a, 1988). As a result of this difference in egg size, cancrids have much higher fecundity per brood than geryonids of comparable body size (by nearly an order of magnitude, about 1 000 000 eggs in cancrids versus about 225 000 eggs in geryonids). Cancrid eggs, however, are not unusually small, and many species in other families have eggs which are of similar size (e.g. Grapsidae, Ocypodidae, and Xanthidae) or smaller (e.g. Portunidae and Pinnotheridae) (Hines 1986a). Although the fecundities of several cancrids (e.g. *C. antennarius*, *C. anthonyi*, and *C. pagurus*) are among the highest reported for a brachyuran species, much of the high fecundity results from their large body size. Some other species of crabs (e.g. *Callinectes sapidus* with 1–2 million eggs; Hines 1982) produce comparable numbers of eggs per brood at smaller body size.

Thus, reproductive strategies in the Cancridae appear to have evolved in direct covariation with body size, which is in turn related to variation in growth patterns in the life histories of the species (see Orensanz and Gallucci 1988). The biogeographic restriction of cancrids to cold temperate and boreal zones (Mackay 1943; Nations 1975, 1979) apparently restricts the brooding activity in most species to a winter–spring season that results both in larval release during the spring peak of plankton production and in settlement during warm summer temperatures optimal for growth. This seasonal restriction may limit the rate of brood production, and the allometric constraint on brood size by body space for yolk accumulation (Hines 1982, 1988) further restricts variation in reproductive output. Variation in fecundity is inherently constrained by a trade-off with egg size (Hines 1982, 1986b). Because the Cancridae apparently have evolved a pattern of covariation in reproductive traits which differs from the Geryonidae, detailed analyses of reproductive patterns in other families radiating into other ecological zones will be necessary to understand fully the evolutionary constraints and variables of brachyuran reproductive strategies.

Acknowledgments

I thank the following people for collecting samples of ovigerous crabs: Dan Wickham for *C. magister*, Steve Sulkin for *C. oregonensis*, Chris Toole for *C. antennarius* and *C. productus*, John Born and Jeff Shields for *C. anthonyi*, Jay Carroll for *C. antennarius*, Stuart Sherburne for *C. borealis* and *C. irroratus*, Richard Emlet for *C. gracilis*, and Donald Cameron for *C. pagurus*. Janet Barnes and Deborah Pal-

mer assisted with egg counts. Richard Hartnoll, Peter Schwinghamer, and an anonymous reviewer provided helpful comments on the manuscript. This project was funded in part by the Fluid Research Fund of the Smithsonian Institution and the Smithsonian Marine Station at Link Port.

References

ANDERSON, W. R., AND R. R. FORD. 1976. Early development, growth and survival of the yellow crab *Cancer anthonyi* Rathbun (Decapoda, Brachyura) in the laboratory. Aquaculture 7: 267–279.

BENNETT, D. B. 1974. Growth of the edible crab (*Cancer pagurus* L.) off southwest England. J. Mar. Biol. Assoc. U.K. 54: 803–823.

BIGFORD, T. E. 1979. Synopsis of biological data on the rock crab, *Cancer irroratus* Say. NOAA Tech. Rep. NMFS Circ. 426, FAO Synop. No. 123: 26 p.

BUTLER, T. E. 1961. Growth and age determination of the Pacific edible crab *Cancer magister* Dana. J. Fish. Res. Board Can. 18: 873–889.

CAMPBELL, A., AND M. D. EAGLE. 1983. Size at maturity and fecundity of rock crabs, *Cancer irroratus*, from the Bay of Fundy and southwestern Nova Scotia. Fish. Bull. 81: 357–362.

CARPENTER, R. K. 1978. Aspects of growth, reproduction, distribution and abundance of the jonah crab (*Cancer borealis* Stimpson) in Norfolk Canyon and the adjacent slope. M.A. dissertation, University of Virginia, Charlottesville, VA. 69 p.

CARROLL, J. C. 1982. Seasonal abundance, size composition, and growth of rock crab, *Cancer antennarius* Stimpson, off central California. J. Crustacean Biol. 2: 549–561.

CLEAVER, R. C. 1949. Preliminary results of the coastal crab (*Cancer magister*) investigation. Wash. State Dep. Fish. Biol. Rep. 49A: 47–82.

COLLIER, P. C. 1983. Movement and growth of post-larval Dungeness crabs, *Cancer magister*, in the San Francisco area. *In* P. W. Wild and R. N. Tasto [ed.] Life history, environment, and mariculture studies of the Dungeness crab, *Cancer magister*, with emphasis on the central California fishery resource. Fish Bull. (Calif.) 172: 125–133.

DEEVEY, E. S. 1947. Life tables for natural population of animals. Q. Rev. Biol. 22: 283–314.

EDWARDS, E. 1979. The edible crab. Fishing News Books Ltd., Farnham, Surrey.

GARTH, J. S., AND D. P. ABBOTT. 1980. Brachyura: the true crabs, p. 594–630. *In* R. H. Morris, D. P. Abbott, and E. C. Haderlie [ed.] Intertidal invertebrates of California. Stanford University Press, Stanford, CA.

GUTIERREZ, J. M., AND O. R. ZUNIGA. 1976. *Cancer setosus* Molina en la Bahia de Mejillones L sur (Crustacea, Decapoda, Brachyura). Rev. Biol. Mar. 16: 1–25.

HAEFNER, P. A. JR. 1976. Distribution, reproduction and moulting of the rock crab, *Cancer irroratus* Say, 1917, in the mid-Atlantic Bight. J. Nat. Hist. 10: 377–397.

1977. Aspects of the biology of the jonah crab, *Cancer borealis* Stimpson, 1859 in the mid-Atlantic Bight. J. Nat. Hist. 11: 303–320.

HAEFNER, P. A., JR., AND W. A. VAN ENGEL. 1975. Aspects of molting, growth and survival of male rock crabs, *Cancer irroratus*, in Chesapeake Bay. Chesapeake Sci. 6: 253–265.

HANCOCK, D. A., AND E. EDWARDS. 1967. Estimation of annual growth in the edible crab (*Cancer pagurus* L.). J. Cons. Int. Explor. Mer 31: 246–264.

HANKIN, D., N. DIAMOND, M. MOHR, AND J. IANELLI. 1985. Molt increments, annual molting probabilities, fecundity and survival rates of adult female Dungeness crabs in northern California, p. 189–208. *In* B. R. Melteff [ed.] Proceedings of the symposium on Dungeness crab biology and management. Lowell Wakefield Symposia on Fisheries, Univ. Alaska, Alaska Sea Grant Rep. No. 85-3.

HINES, A. H. 1982. Allometric constraints and variables of reproductive effort in brachyuran crabs. Mar. Biol. 69: 309–320.

1986a. Larval patterns in the life histories of brachyuran crabs (Crustacea, Decapoda, Brachyura). Bull. Mar. Sci. 39: 444–466.

1986b. Larval problems and perspectives in life histories of marine invertebrates. Bull. Mar. Sci. 39: 506–525.

1988. Fecundity and reproductive output in two species of deep-sea crabs, *Geryon fenneri* and *G. quinquedens* (Decapoda: Brachyura). J. Crustacean Biol. 8: 557–562.

INGLE, R. W. 1981. The larval and post-larval development of the edible crab, *Cancer pagurus* Linnaeus (Decapoda: Brachyura). Bull. Brit. Mus. Nat. Hist. (Zool.) 40: 211–236.

KNUDSEN, J. W. 1964. Observations of the reproductive cycles and ecology of the common Brachyura and crablike Anomura of Puget Sound, Washington. Pac. Sci. 18: 3–33.

KROUSE, J. S. 1976. Size composition and growth of young rock crab, *Cancer irroratus*, on a rocky beach in Maine. Fish. Bull. 74: 949–954.

MACKAY, D. C. G. 1942. Relative growth of the European edible crab, *Cancer pagurus*: I. Growth of the carapace. Growth 6: 251–248.

1943. Temperature and the world distribution of crabs of the genus *Cancer*. Ecology 24: 113–115.

MACKAY, D. C. G., AND F. W. WEYMOUTH. 1935. The growth of the Pacific edible crab, *Cancer magister* Dana. J. Biol. Board Can. 1: 191–212.

MELTEFF, B. R. [ED.] 1985. Proceedings of the symposium on Dungeness crab biology and management. Lowell Wakefield Symposia on Fisheries, Univ. Alaska, Alaska Sea Grant Rep. No. 85-3.

NATIONS, D. 1975. The genus *Cancer* (Crustacea: Brachyura): systematics, biogeography and fossil record. Sci. Bull. Nat. Hist. Mus. Los Angeles Cty. 23: 1–104.

1979. The genus *Cancer* and its distribution in time and space. Bull. Biol. Soc. Wash. 3: 153–187.

ORENSANZ, J. M., AND V. F. GALLUCCI. 1988. Comparative study of postlarval life-history schedules in four sympatric species of *Cancer* (Decapoda: Brachyura: Cancridae). J. Crustacean Biol. 8: 187–220.

POOLE, R. L. 1966. Preliminary results of the age and growth study of the market crab (*Cancer magister*) in California: the age and growth of *Cancer magister* in Bodega Bay. Mar. Biol. Assoc. India 2: 553–567.

REILLY, P. N., AND S. B. SAILA. 1978. Biology and ecology of the rock crab, *Cancer irroratus* Say, 1817, in southern New England waters (Decapoda, Brachyura). Crustaceana 34: 121–140.

SASTRY, A. N. 1977. The larval development of the jonah crab, *Cancer borealis* Stimpson, 1859, under laboratory conditions (Decapoda, Brachyura). Crustaceana 32: 290–303.

SCARATT, D. J., AND R. LOWE. 1972. Biology of rock crab (*Cancer irroratus*) in Northumberland Strait. J. Fish. Res. Board Can. 29: 161–166.

SHIELDS, J. D. 1987. The dynamics and mechanisms of egg mortality in *Cancer anthonyi* (Decapoda). Ph.D. dissertation, University of California, Santa Barbara, CA. 177 p.

1991. The reproductive ecology and fecundity of *Cancer* crabs. *In* A. Wenner and A. M. Kuris [ed.] Crustacean Issues, Crustacean Egg Production 7. (In press)

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. The principles and practice of statistics in biological research. 2nd ed. Freeman Co., New York, NY. 859 p.

TRASK, T. 1970. A description of laboratory reared larvae of *Cancer productus* Randall (Decapoda, Brachyura) and a comparison to larvae of *Cancer magister* Dana. Crustaceana 18: 133–146.

WICKHAM, D. E. 1979a. Predation by *Carcinonemertes errans* on eggs of the Dungeness crab, *Cancer magister*. Mar. Biol. 55: 45–53.

1979b. *Carcinonemertes errans* and the fouling and mortality of eggs of the Dungeness crab, *Cancer magister*. J. Fish. Res. Board Can. 36: 247–257.

1980. Aspects of the life history of *Carcinonemertes errans* (Nemertea: Carcinonemertidae), an egg predator of the crab *Cancer magister*. Biol. Bull. 159: 247–257.

WILD, P. W., AND R. N. TASTO [ED.] 1983. Life history, environment, and mariculture studies of the Dungeness crab, *Cancer magister*, with emphasis on the central California fishery resource. Fish Bull. (Calif.) 172.

Abundance and Distribution of Narwhals (*Monodon monoceros*) in Northern Hudson Bay

Pierre R. Richard

Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6, Canada

Richard, P. R. 1991. Abundance and distribution of narwhals (*Monodon monoceros*) in northern Hudson Bay. Can. J. Fish. Aquat. Sci. 48: 276–283.

Aerial surveys for narwhals (*Monodon monoceros*) were conducted in August 1981, in July 1982, 1983, and 1984, and in March 1983 in the Repulse Bay area between Roes Welcome Sound and Lyon Inlet, north of Southampton Island. About 1300 narwhals were estimated to occupy waters between northern Roes Welcome Sound and Lyon Inlet in July 1982, 1983, and 1984. Narwhals were found throughout the study area but were more concentrated in three areas: northern Roes Welcome Sound, southern Frozen Strait, and, in 1984, Lyon Inlet. Only four narwhals were observed during August 1981 reconnaissance surveys of these areas. No narwhals were seen during the March 1983 survey of Roes Welcome Sound and leads of Frozen Strait. A winter concentration of narwhals observed by others in eastern Hudson Strait was of similar size to that which we observed in July, suggesting that they could be the same stock. If this is so, the northern Hudson Bay narwhals could be distinct from the stock that inhabits Davis Strait and Baffin Bay.

Des inventaires aériens ont été effectués en août 1981, en juillet 1982, 1983 et 1984 et en mars 1983 dans la région de la baie Repulse entre le détroit de Roes Welcome et la baie de Lyon, au nord de l'île Southampton. On estime à environ 1 300 le nombre de narvals (*Monodon monoceros*) qui occupaient en juillet 1982, 1983 et 1984 les eaux situés entre la partie nord du détroit de Roes Welcome et la baie de Lyon. On a observé des narvals dans presque toute l'aire d'étude mais ils étaient plus concentrés en trois endroits: la partie nord du détroit Roes Welcome, la partie sud du détroit Gelé et, en 1984, la baie Lyon. En août 1981, on a observé seulement quatre narvals durant des vols de reconnaissance aérienne couvrant ces mêmes endroits. Aucun narval n'a été vu durant l'inventaire de mars 1983 du détroit de Roes Welcome et des fentes dans la banquise du détroit Gelé. D'autres chercheurs ont noté la présence en hiver dans la portion est du détroit d'Hudson d'une concentration de narvals de nombre semblable à celle que nous avons observé en juillet dans le nord de la Baie d'Hudson. Cela suggère que la population du nord de la baie d'Hudson pourrait être un stock distinct de celui qui occupent le détroit de Davis et la baie de Baffin.

Received December 4, 1989
Accepted August 10, 1990
(JA384)

Reçu le 4 décembre 1989
Accepté le 10 août 1990

Narwhals (*Monodon monoceros*) have long been known to occur in northern Hudson Bay (Lyon 1824; Sutton and Hamilton 1932; Degerbøl and Freuchen 1935; Mansfield et al. 1975) but descriptions of their seasonal distribution have been limited to a few anecdotal accounts from local residents (Freeman 1976). Until now, there had been no attempt to estimate the number of narwhals which occupy that area. Available information on narwhal distribution and movements is also insufficient to determine if northern Hudson Bay narwhals are a part of the large stock that inhabits Davis Strait and Baffin Bay or if they constitute a separate stock (Mitchell and Reeves 1981). They are presently managed as a separate stock but growing interest in this resource by native hunters of the Keewatin emphasizes the need to determine its size and distribution so that managers can establish sustainable harvest limits.

The objectives of this study were to estimate the size of the population of narwhals during their summer concentration in northern Hudson Bay and secondarily to determine whether they winter in that area, thereby forming a geographically isolated stock.

Results of 4 yr of surveys (1981–84) are presented. They include a combination of reconnaissance and census surveys: five summer and one winter surveys. Estimates of narwhal abundance are given for each census survey and the distribution of narwhals is described. Finally, we discuss stock delineation while comparing our distributional results with published information and local accounts.

Methods

Before our surveys, we sought advice from Repulse Bay hunters on the choice of areas and the timing of summer census surveys of narwhals. Our summer study area is bounded on the south by 65°00′N, the west by 87°30′W, the north by 66°30′N, and the east by 83°00′W. It includes Repulse Bay, Frozen Strait, Gore Bay, Lyon Inlet, the northern part of Roes Welcome Sound, and Duke of York Bay (Fig. 1). Hunters were of the opinion that the peak of narwhal summer occupation in these areas coincides with the early part of the open-water season which usually takes place during late July. At that time of year, the surveyed areas except those immediately adjacent to Foxe Channel were almost entirely clear of ice (<2/10 ice cover).

In 1981, we flew a reconnaissance of the whole study area and of the coastlines of Southampton Island on 19–21 August (Fig. 2A). In the years that followed (1982–84), we flew all our summer surveys between 23 and 29 July (Fig. 3). Breakup generally took place a week or two before the date of surveys

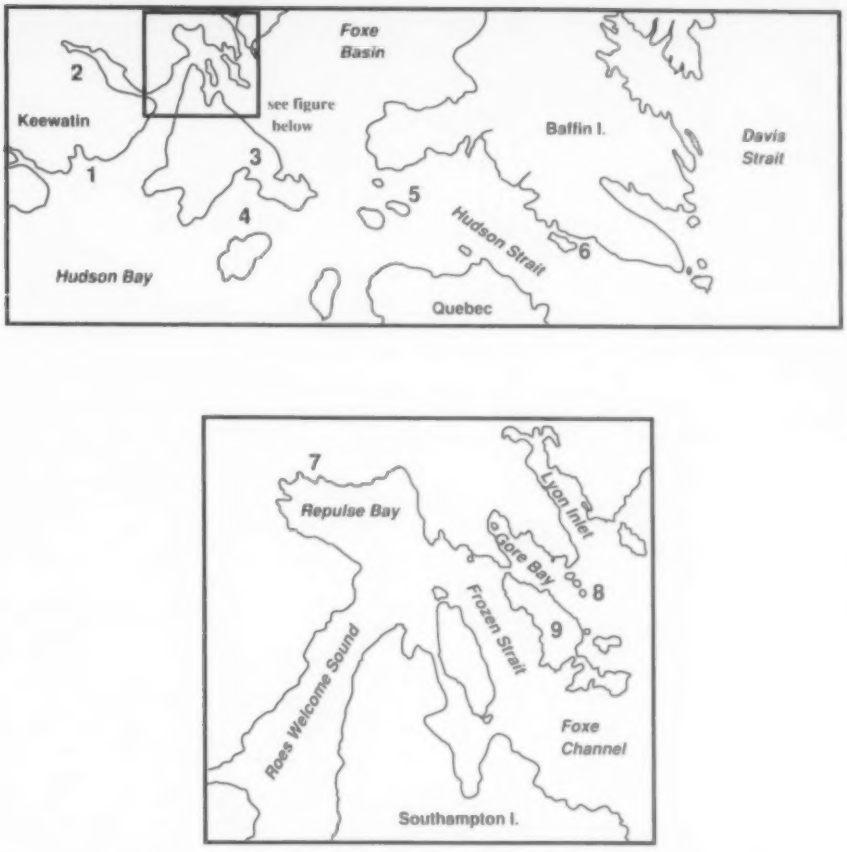


FIG. 1. Areas discussed in text: 1, Cape Fullerton; 2, Wager Bay; 3, hamlet of Coral Harbour; 4, South Bay; 5, Cape Dorset; 6, Lake Harbour; 7, hamlet of Repulse Bay; 8, Sturges-Bourne Island; 9, Vansittart Island.

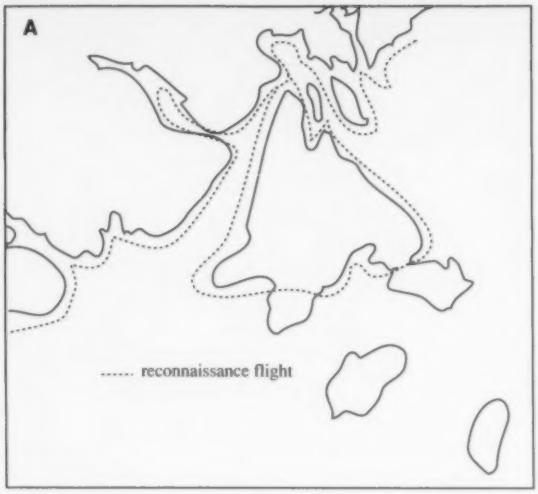
and most of the areas surveyed had open water or sparsely distributed ice. Foxe Channel, on the other hand, generally had high concentrations of ice (>7/10 ice cover). Our 1983 winter survey area covered the open leads of Frozen Strait and Roes Welcome Sound north of 64°00′N (Fig. 2B) and took place on 18–20 March to take advantage of the lengthening daylight. It also allowed comparison with the results of a prior survey in March 1981 which covered much of the same area (Finley et al. 1982).

The 1981 reconnaissance survey (Fig. 2A; Table 1) was flown in a De Havilland Beaver with one observer in the right front seat and the other in the left rear. Navigation was by dead-reckoning using land forms. All other surveys were flown in a De Havilland Twin Otter. The July 1982 and March 1983 visual census surveys (Fig. 2B, 3A; Table 1) were done with observers seated directly behind wing struts, one on each side of the aircraft. Struts and windows were marked to delimit a survey strip (Table 1) which we calibrated by flying at survey altitude over reference marks spaced at known lengths. Flight position was determined by pilot and navigator with Omega Navigation and was regularly checked against landmarks. Air speed and altitude were maintained at 185 km·h^{-1} and 305 m during visual surveys.

For photographic surveys, we used a Linhof Aeroteknica (format 95 × 120 mm) with a 150-mm lens and Kodak Ektachrome transparency film in 1982–83 (Fig. 3B, 3C; Table 1) and a Wild-Leitz RC8 (225 × 225 mm) with a 153-mm lens and Kodak aerocolor 2445 negative film in 1984 (Fig. 3D; Table 1). Cameras were triggered by an intervalometer at settings calculated to obtain adjoining frames. Variations in aircraft speed resulted in occasional but small overlap or gaps between frames. Position was determined by Omega Navigation in 1982 and 1983 and Inertial Navigation in 1984, verified and updated by land references. Air speed and altitude were maintained at 185 km·h^{-1} and 914 m.

The 1982 random survey was flown to obtain an unbiased estimate of the variance in narwhal density over the survey area. It was planned by randomly selecting start points and flight directions for 8-km east–west transects. Each day a new set of transects was selected and transects were flown in sequence from northwest to southeast. In 2 d, we surveyed a total of 55 transects (Fig. 3B; Table 1). This survey was also used to test the feasibility of censusing narwhals photographically.

Subsequently, we used systematic sampling because it is more operationally convenient and less expensive. It is also more efficient than random sampling as a means of mapping distribution (Caughley 1977) and more precise to estimate pop-



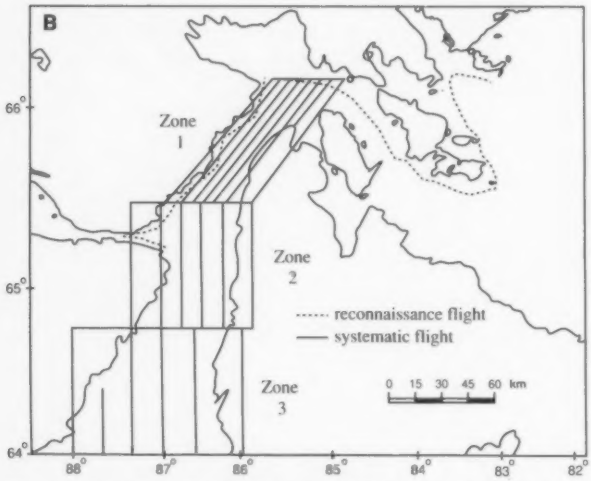


FIG. 2. Survey lines for the (A) August 1981 reconnaissance survey and (B) March 1983 systematic surveys.

ulation size when sample units are serially correlated (Kingsley and Smith 1981) or when sample units are unequal in size (Cochran 1977) which was the case in our surveys.

We also chose, thereon, to conduct photographic census surveys because narwhals could be imaged quite clearly. There were no problems of reliability due to observer fatigue, boredom, and counting accuracy such as are encountered in visual surveys. Also, narwhal visibility bias with distance from the flight line is much reduced by vertical photography. The 43–90° angles of the camera from photo edge to photo center allow an improved water penetration across the image plane. Variation in image coverage due to airplane roll can be almost eliminated by maintaining the camera level during operation whereas there is no way of correcting strip width variation during visual surveys.

In 1983, we surveyed the study area almost entirely with the Linhof camera on 26–27 July, but because of camera malfunction towards the end of the survey, Gore Bay and Lyon Inlet were not flown. On 27 and 29 July 1984, we flew a second systematic photosurvey with the larger format Wild-Leitz camera. That survey covered Gore Bay and Lyon Inlet but not Duke of York Bay because we had seen very few narwhals there in the previous 3 yr.

The film was scanned with a Nikon SMZ-1 dissecting scope on a Richards GFL-940-MC light table by a single observer who recorded all narwhals and "blobs" which could be narwhals but whose identity was questionable. Frames with questionable "blobs" were later reexamined by three observers and were retained as narwhals if at least two observers agreed on that classification.

Estimates of the total visible population were derived from the three July systematic census surveys. The number of narwhals and the surveyed area were totalled for each transect. In the case of photosurveys, the area of each transect was the sum of frame areas. Land or fog encountered on the strip or photos was removed from the area total. Transect counts and areas were summed and the ratio of total count over total area gave a mean density. Estimates of narwhal population were obtained by multiplying the narwhal density by the total area surveyed. In cases where the survey area was divided into zones, estimates from all zones were summed to obtain an estimate for the whole survey area.

During surveys at 305 m, Norton and Harwood (1985) found that sightability of beluga (*Delphinapterus leucas*) dropped sharply beyond 600 m of their flight line. Seen from the air, narwhals are of similar length and only slightly darker than beluga. Although we do not have data to verify this, we assumed that, for narwhal, visibility with distance is similar or worse that that of beluga. We therefore used a swath of 600 m in our calculation of the visual survey estimate, instead of the 800-m strip actually scanned by observers. By applying this correction, we hope to avoid underestimating the population size of narwhals seen on the visual survey. We did not apply correction factors in any of the estimates to compensate for submerged animals missed during the flights; factors applicable for this kind of survey are not yet available (Smith and Hammill 1986).

Methods described by Kingsley et al. (1985) were used to obtain estimates of variance of density, except that we did not apply any weighting to the counts for meridian convergence in north–south transects or to the surveyed area for ice cover. Meridian convergence is minimal in our survey area and ice cover was not removed in area calculations to avoid overestimating narwhal density in ice. Confidence limits of survey density were calculated following Smith et al. (1985) by assuming that true mean and sampling variance are proportional and that the constant of proportionality can be estimated from the sample's variance–mean ratio. The confidence limits R_L of whale density were thus obtained by

$$(1)\quad R_L - t\sqrt{\frac{S^2{}_2 R_L}{\hat{R}}} = \hat{R}$$

which solved for R_L gives

$$(2)\quad \sqrt{R_L} = \frac{\left(\frac{-t\,S_2}{\sqrt{\hat{R}}} \pm \sqrt{\frac{t^2 S^2{}_2}{\hat{R}} + 4\hat{R}}\right)}{2}$$

where R_L are the confidence limits of the density estimate R, t is the critical point in Student's t distribution, and S_2^2 is the

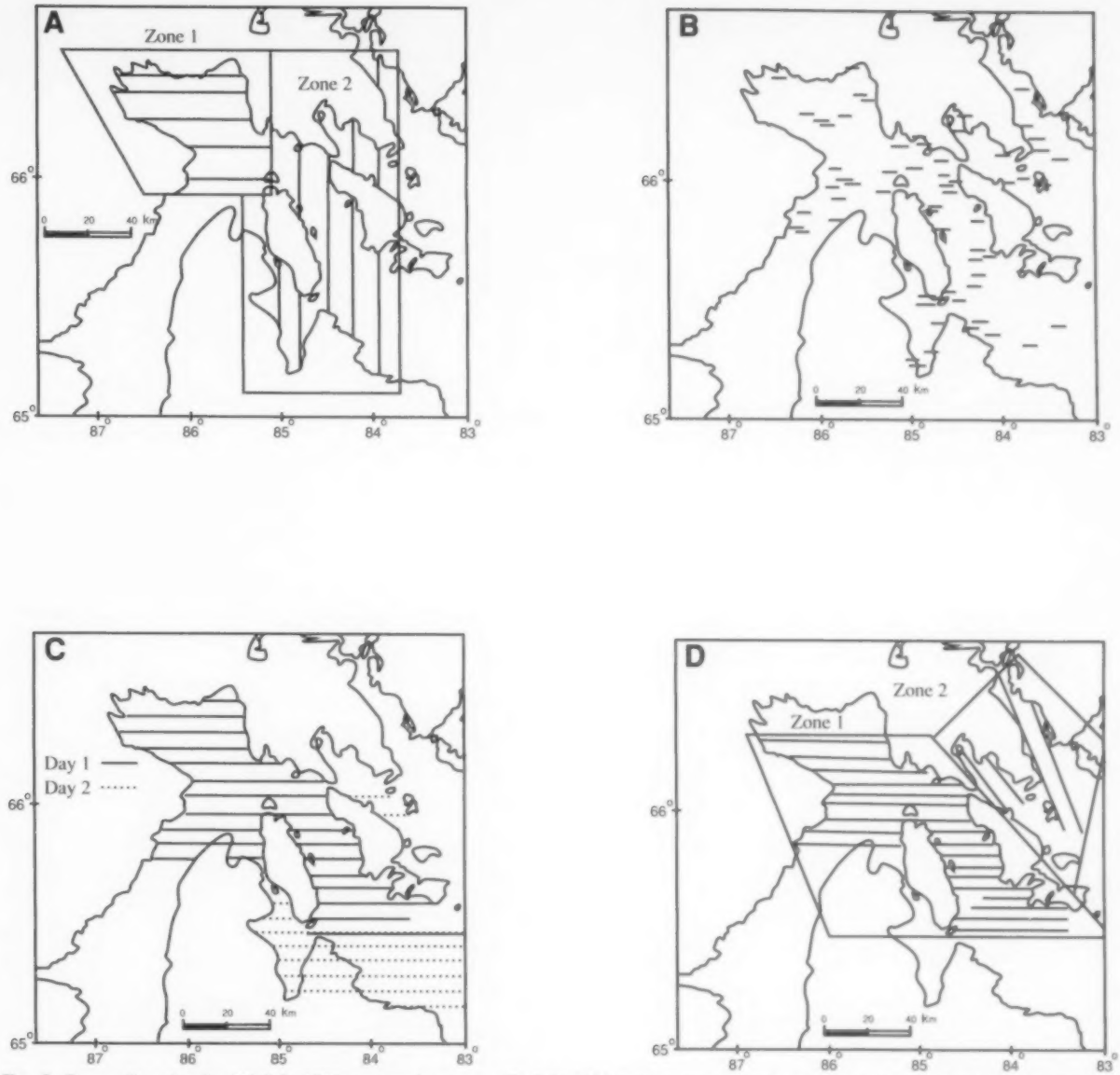


FIG. 3. Survey lines for the (A) July 1982 systematic survey, (B) July 1982 random survey, and (C and D) July 1983 and 1984 systematic surveys.

variance estimate given by Kingsley et al. (1985) for systematic samples. Minimum and maximum population estimates for each survey were calculated by multiplying the mean density estimate and its upper and lower confidence limits by the total census area.

For the 1984 survey, we also obtained confidence limits for both zones combined by calculating the confidence distribution of the population estimate of each zone using the above method to calculate confidence limits and by doing a numeric convolution (Kotz et al. 1982) of these two distributions to obtain the confidence distribution of the combined estimate.

The effectiveness of our systematic surveys was evaluated by calculating the ratio of two measures of clumping given by Kingsley et al. (1985):

$$(3)\quad C_1 = \frac{S^2{}_1 \sum\sum t_{ij}}{R} \quad \text{or} \quad C_1 = E^2{}_1 \sum\sum t_{ij}$$

$$(4)\quad C_2 = \frac{S^2{}_2 \sum\sum t_{ij}}{R} \quad \text{or} \quad C_2 = E^2{}_2 \sum\sum t_{ij}$$

where S_1^2 is the variance estimate given by Cochran (1977) for random samples and S_2^2 is the variance estimate given by Kingsley et al. (1985) for systematic samples, E_1^2 and E_2^2 are the corresponding squares of the error coefficients of variation, $\Sigma\Sigma t_{ij}$ is the total narwhal count over j frames or intervals per transect and over i transects, and R is the estimated narwhal density.

If narwhals were randomly and independently distributed, the expected value of both estimates of variance $S_1{}^2$ and $S_2{}^2$

TABLE 1. Methods of Northern Hudson Bay aerial narwhal surveys, 1981–84.

Survey date(s) (Fig. No.)	Survey design	Method of count	Altitude (photo scale)	Transect spacing	Strip width	Flight path
18–25 August 1981 (Fig. 2A)	Reconnaissance	Visual, two observers[a]	305 m	Variable[b]	0.8 km on each side	Variable[b]
23 July 1982 (Fig. 3A)	Systematic	Visual, two observers[a]	305 m	Zone 1: 19 km Zone 2: 11 km	0.8 km on each side	Zone 1: 90–270° Zone 2: 0–180°
24–25 July 1982 (Fig. 3B)	Random	Photographic, 96 × 122 mm	914 m (1/6054)	Variable[c]	0.45 km	90–270°
18–21 March 1983 (Fig. 2B)	Systematic	Visual, two observers[a]	305 m	Zone 1: 7.5 km Zone 2: 13 km Zone 3: 19 km	0.8 km on each side	Zone 1: 45–225° Zone 2: 0–180° Zone 3: 0–180°
26–27 July 1983 (Fig. 3C)	Systematic	Photographic, 96 × 122 mm	914 m (1/6054)	9.4 km	0.45 km	90–270°
27 and 29 July 1984 (Fig. 3D)	Systematic	Photographic, 225 × 225 mm	914 m (1/6000)	5.5 km	1.34 km	90–270°

[a]One left and one right observer; narwhal counts totalled every 5-min interval.
[b]Departure leg: 3 km offshore; return leg (when on same track): 8 km offshore.
[c]Transects start point and direction (east or west) drawn randomly; transect length 8 km.

would be equal, and the error coefficient of variation would be equal to $1/\sqrt{\Sigma\Sigma t_{ij}}$ (Kingsley et al. 1985). Clump factors greater than unity (e.g. 2 or more) indicate nonrandom nonindependent narwhal distributions. The first clump factor (C_1) is an index of narwhal clumping over the whole survey area, while the second (C_2) is an index of the degree of clumping between adjacent transects. If the ratio C_1/C_2 is greater than 1 (e.g. 2 or more), which means that adjacent transects have similar densities when compared with distant ones, stratification of the survey area into strata of uniform density could be effective in reducing sampling variance and consequently increasing precision of the estimates.

When stratification is ineffective, the only pratical way of reducing variance is to increase coverage. Following Smith et al. (1985) we used results of the July 1984 surveys and estimated for the study area (A) the percent coverage (P) needed for a desired level of precision, or error coefficient of variation (E), given known mean whale density (R) and clump factor C_1. From equations (3) and (4), we get

$$(5)\quad E^2 = \frac{C}{\sum\sum t_{ij}}$$

and density is given by

$$(6)\quad R = \frac{\sum\sum t_{ij}}{a}$$

where a is the area sampled. Percent coverage (P) can then be estimated by

$$(7)\quad P = \frac{C}{E^2RA}.$$

This method is useful to determine what degree of precision can be expected in later surveys, all conditions being equal, if greater coverage could be afforded.

To summarize the July distribution of narwhals, we used an index of abundance based on block densities (Kenney and Winn 1986). The study area was divided into blocks of 5 min of latitude and 15 min of longitude. For each block, we calculated the area covered by each survey and the corresponding number of narwhals seen. Results were totalled for each block and block densities were calculated. Densities were then smoothed by averaging each block with the weighted values of adjacent blocks. Densities of horizontal and vertical adjacent blocks were weighted 0.5, diagonal blocks 0.333, and the target block 1.0. Contour lines of equal densities were then overlaid on a map of the survey area.

Results

In August 1981, only four narwhals were seen in the study area during reconnaissance flights. They were located near Sturges-Bourne Islands (Fig. 1). Further reconnaissance flights of the north and south coasts of Southampton Island and of both shores of Roes Welcome Sound did not result in any more narwhal sightings in August.

In March 1983, we did not see any narwhals in Roes Welcome Sound or in the leads of Frozen Strait and Lyon Inlet (Fig. 2B). Roes Welcome Sound had extensive areas of open water in the week before the survey, but during the survey, a combination of calm and cold weather caused a thin transparent layer of new ice to cover most of the area. Breathing holes were observed frequently in this new ice but they could have been made by other species, such as seals, which were frequently seen during this survey.

Estimates for the four July surveys (Fig. 3) range between 1038 and 1517 narwhals with varying degree of precision (Table 2). In all surveys, narwhals were clumped in distribution and this resulted in large estimated error variances. The large estimates of variance could not be improved by stratification because no homogeneous areas could be defined within survey areas, adjacent transect clumping (C_1) being more or less equal than overall clumping (C_2) (Table 2). The census area was divided into zones for logistical reasons only.

Census estimate and precision (i.e. coefficient of variation) was lowest during the visual survey because it had the lowest narwhal densities, despite the strip width correction to 600 m, and because survey coverage was low. Precision was improved in 1983, although only slightly, by increasing the number of

TABLE 2. Population estimates and sample statistics of July census surveys in northern Hudson Bay, 1982–84.

Survey date(s)	Zone	% coverage	Mean density (narwhals·km^{-2})	Mean estimate	Confidence limits (95% prob. C.L.) [90% prob. C.L.]	Coefficient of variation (%)	Ratio C_1/C_2
23 July 1982	1	11.2	0.18	511	(181–1449) [227–1154]	39	1.4
23 July 1982	2	7.4	0.1	527	(106–2629) [146–1898]	64	0.8
23 July 1982	Total	8.7	0.14	1038			
24–25 July 1982		3.1	0.12	1309	(583–2861) [661–2523]	38	n/a
26–27 July 1983		10.4	0.19	1517	(724–3175) [820–2806]	36	0.9
27 July 1984	1	16.8	0.14	825	(441–1546) [491–1388]	30	1
29 July 1984	2	11.0	0.25	521	(201–1345) [255–1063]	31	1.2
27–29 July 1984	Total	15.6	0.17	1355	(910–2100) [1000–1900]		

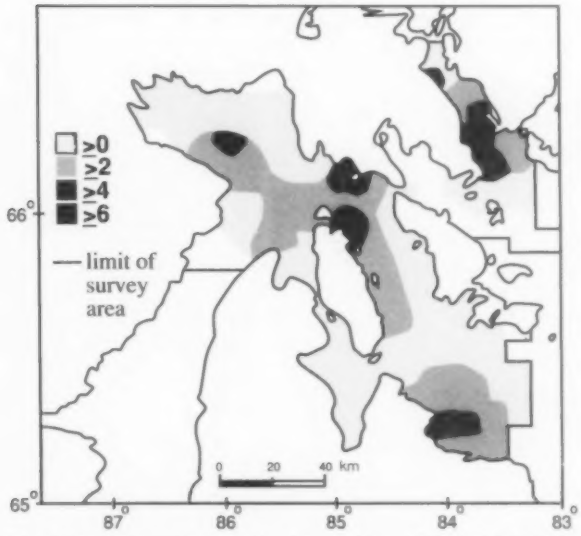


FIG. 4. Average narwhal densities (narwhals·km^{-2}) during July 1982–84 surveys.

transects and by the choice of transect lines that were more uniform in length. The greatest precision was obtained during the 1984 survey when we increased coverage to 16.8% in zone 1 (E.C.V. = 30%) and encountered a relatively high and uniform density in zone 2 (E.C.V. = 31%). The combined two-zone estimate for this survey is 1355 narwhals with 95% confidence limits of 910–2100 narwhals and 90% confidence limits of 1000–1900.

During these July surveys, narwhals were distributed over almost all the survey area, with the exception of Gore Bay and the area along the northeast shore of Vansittart Island (Fig. 4). High density areas were the northern end of Roes Welcome Sound, the southern half of Frozen Strait, and Lyon Inlet.

The Lyon Inlet concentration (Fig. 4) was observed only in 1984 (Fig. 3D, zone 2) when we recorded a relatively high density of narwhals (Table 2). This observation is in contrast with the absence of narwhals in both the 1982 visual and photographic transects over Lyon Inlet.

Discussion

During our surveys, the water clarity and low ice cover allowed us to see narwhals several metres below the surface, but animals making deep dives would be missed and the estimates provided are therefore negatively biased.

Obtaining a higher precision on these estimates would be difficult. Stratification would not be efficient in reducing the variance because there are no indications that the study area can be predictably divided into areas of high narwhal density and areas of low density.

The 1984 survey gave the narrowest confidence intervals, largely because it had the highest survey coverage (17%). Cost and logistic constraints prohibit the increase of survey coverage beyond that attained in 1984. To reduce the coefficient of variation by half, coverage in zones 1 and 2 should be respectively four and five times greater than it was in 1984. Even doubling the coverage would only reduce the coefficient of variation by one third. Such tight grids would be difficult to fly without navigational errors and transect overlap, which would bias the estimate of the error variance.

Kingsley and Smith's (1981) method of estimating true population variance better reflects the precision of systematic surveys on natural populations than the traditional sample estimate (Caughley 1977). Yet, they showed that it still overestimates the error variance somewhat, especially with low coverage surveys. Consequently, the use of a 95% critical level seems unnecessarily conservative when deriving confidence limits, and the less stringent 90% confidence level should probably be used for management decisions.

The northern Hudson Bay summer population was previously reported to be small compared with the High Arctic summer population (Mansfield et al. 1975; Mitchell and Reeves 1981). Mean densities recorded during our July surveys are generally lower than those observed in the High Arctic both in

July and August (Fallis et al. 1983; Smith et al. 1985). The High Arctic population is estimated to number approximately 20 000 animals (Strong 1988), while our mean estimates for the July survey area in northern Hudson Bay vary between 1000 and 1500 over the four surveys.

Our coverage in July was limited to areas of open water or low ice cover (<2/10). We did not survey Foxe Basin because of its considerable size and the difficulty of seeing narwhals in the high concentrations of ice (>9/10 cover) it contained in July. The absence of narwhals in Lyon Inlet in July 1982 compared with the high density observed there in July 1984 suggests that the distribution of narwhals is variable. We observed narwhals again at the mouth of Lyon Inlet during a reconnaissance survey on 26 July 1989 (P. Richard, unpubl. data). This indicates that their presence in Lyon Inlet is more than occasional. Anecdotal hunter observations confirm this view.

In addition to sampling error, some of the variation in population estimates could be due to changes in distribution as narwhals move in or out of the study area. The July study area was delimited using native intelligence on narwhal distribution, but other areas could also be used.

For example, Sutton and Hamilton (1932) have mentioned that "Wager Inlet is said to be a common haunt for narwhals" but they give no detail to substantiate this remark. The occurrence of narwhals in Wager Bay or in the southern portion of Roes Welcome Sound was not mentioned in any of our discussions with hunters. Two narwhals were sighted by a research team in Wager Bay in August 1976 (Anonymous 1977) but this appears to be the only recorded sighting there.

Sutton and Hamilton (1932) also report a few observations of narwhals in South Bay on the south shore of Southampton Island but added that "they are known to be rare there." Recent July reconnaissance surveys in 1989 found no narwhals in that area (P. Richard, unpubl. data). Narwhals are also rare in Hudson Strait in summer (Low 1906; Soper 1944; Freeman 1976). Local observations and catch records indicate that their occurrence in the vicinity of Cape Dorset and Lake Harbour is more or less episodic and usually in the spring and fall (Soper 1944; Mansfield et al. 1975; Freeman 1976).

I conclude that narwhals are not usually found in summer in the above-mentioned areas. One possible exception is that of Foxe Channel whose heavy ice in summer forbids travel by locals and consequently observations of narwhals. Systematic surveys should be undertaken to determine if narwhals do make use of Foxe Channel at that time of year.

Until recently, there was very little information on the winter distribution of narwhals, and the stock identity of the northern Hudson Bay population was an open question (Mitchell and Reeves 1981). Sutton and Hamilton (1932) have suggested that narwhals "occur the year round wherever water is open" in northern Hudson Bay. The Roes Welcome Sound polynya was considered a probable wintering area for Hudson Bay marine mammals (Stirling and Cleator 1981). March surveys in 1981 (Finley et al. 1982) and 1983 (this study) failed to locate any narwhal there or in the leads of Frozen Strait, but on 11 March 1988, a group of narwhals was observed by a local hunter near Cape Fullerton (64°00′N, 88°40′W) at the south end of the Roes Welcome polynya (H. Welch, Department of Fisheries and Oceans, Winnipeg, Man., pers. comm.).

Surveys of eastern Hudson Strait resulted in repeated sightings of narwhals in the months of March–June (McLaren-Marex 1979a, 1979b; McLaren and Davis 1982). I analyzed March survey results of McLaren and Davis (1982). From results of five transects in the eastern portion of Hudson Strait where narwhals were concentrated, I estimate (using the same methods as above) that a total visible population of about 630 (95% C.I: 220–1820) occupied that area in late winter 1981. McLaren and Davis (1982) cautioned that their count was most certainly negatively biased by the difficulty of counting in ice-covered waters. They also reported that a single narwhal was sighted on the western side of Hudson Strait during the same March surveys. This estimate is therefore strongly biased downward and it is reasonable to assume that a concentration of the size of the northern Hudson bay summer concentration winters in eastern Hudson Strait. Another concentration has also been found in Davis Strait (McLaren and Davis 1982) but there is a hiatus of about 300 km between the closest of those sightings and the eastern Hudson Strait concentration.

In contrast with the March sightings, summer surveys of the eastern portion of Hudson Strait have not yielded sightings of narwhals (McLaren-Marex 1979a, 1979b), indicating that they vacate the area in summer. During a series of monthly surveys in eastern Hudson Strait, the last spring sightings of narwhals were made around mid-June and narwhals were not seen again until December (MacLaren-Marex 1979b).

The eastern Hudson Strait winter narwhal population is similar in size to the northern Hudson Bay summer population. It appears to be geographically separated from other winter concentrations and to move out of eastern Hudson Strait in June at a time when narwhals are increasingly seen in northern Hudson Bay. This suggests that the Hudson Strait and northern Hudson Bay populations are one and the same. Other winter sightings of narwhals in northern Hudson Bay could be stragglers trapped behind by ice, or early migrants to the summer areas.

It will be necessary to do mark–recapture or telemetry studies to confirm these hypotheses, but in the mean time, it seems reasonable to assume that the northern Hudson Bay narwhal population is geographically isolated year-round from other narwhal populations. The implication for management is that this population should be managed as an isolated stock of about 1300 narwhals.

Acknowledgements

We are grateful to the many hunters who shared their knowledge of narwhal seasonal distribution. The Repulse Bay Hunters and Trappers Association, in particular, was very helpful in deciding on the choice of areas and the timing of surveys. We also wish to thank the Coral Harbour Hunters and Trappers Associations (HTAs) and the N.W.T. Renewable Resource Department. Many people have contributed by their work, support, and comments to various aspects of this study. I would like to especially thank D. Heyland who convinced us to use aerial photography for surveys of Arctic cetaceans and provided much help and encouragement. Thanks to A. Blouw, M. Kingsley, J. Orr, and G. Yaremchuk for their help in the design, operation, or analysis of surveys. Thanks also to M. Kingsley, A. Kristofferson, R. Moshenko, and T. Strong and an anonymous reviewer for their comments on this manuscript.

References

ANOMYMOUS. 1977. Wager Bay Preliminary Marine Resource Analysis. Report by Marine Studies Section, Parks System Planning Division, National Parks Branch, Parks Canada. vii + 61 p. (Available from the author)

CAUGHLEY, G. 1977. Sampling in aerial survey. J. Wildl. Manage. 41: 605–615.

COCHRAN, W. G. 1977. Sampling techniques. 3rd ed. Wiley, New York, NY. 428 p.

DEGERBØL, M., AND P. FREUCHEN. 1935. Mammals. Report of the Fifth Thule Expedition, 1921–1924. Vol.2(4–5): 278 p. + 1 folding map.

FALLIS, B. W., W. E. KLENNER, AND J. B. KEMPER. 1983. Narwhal surveys and associated marine mammal observations in Admiralty Inlet, Navy Board Inlet and Eclipse Sound, Baffin Island, N.W.T., during 1974–1976. Can. Tech. Rep. Fish. Aquat. Sci. 1211: iv + 20 p.

FINLEY, K. J., G. W. MILLER, M. ALLARD, R. DAVIS, AND C. R. EVANS. 1982. The belugas (*Delphinapterus leucas*) of northern Quebec: distribution, abundance, stock identity, catch history and management. Can. Tech. Rep. Fish. Aquat. Sci. 1123: v + 57 p.

FREEMAN, M. [ED.] 1976. Inuit Land Use and Occupancy Project. Vol. 1. A report prepared by Milton Freeman Research Ltd. for the Department of Indian and Northern Affairs. 263 p.

KENNEY, R. D., AND H. E. WINN. 1986. Cetacean high-use habitats of the northeast United States continental shelf. Fish. Bull. 84: 345–357.

KINGSLEY, M. C. S., AND G. E. J. SMITH. 1981. Analysis of data arising from systematic transect surveys, p. 40–48. *In* F. L. Miller and A. Gunn [ed.] Symposium on census and inventory methods for population and habitats. Proceedings of the Northwest Section, The Wildlife Society, Banff, Alta., April 1980.

KINGSLEY, M. C. S., I. STIRLING, AND W. CALVERT. 1985. The distribution and abundance of seals in the Canadian high Arctic, 1980–82. Can. J. Fish. Aquat. Sci. 42: 1189–1210.

KOTZ, S., N. JOHNSON, AND C. B. READ [ED.] 1982. Encyclopedia of statistical sciences. Vol. 2. John Wiley & Sons, Toronto, Ont. 613 p.

LOW, A. P. 1906. Report on the Dominion Government Expedition to Hudson Bay and the Arctic Islands on board the D.G.S. *Neptune*, 1903–1904. Government Printing Bureau, Ottawa, Ont. Frontis + xvii + 355 p. + 62 plates.

LYON, G. F. 1824. The Private Journal of Captain G. F. Lyon, of H.M.S. *'Hecla,'* during the recent voyage of discovery under Captain Parry. John Murray, London. i-xii + v-viii + 468 p. + 6 plates + folding map.

MANSFIELD, A. W., T. G. SMITH, AND B. BECK. 1975. The narwhal, *Monodon monoceros*, in eastern Canadian waters. J. Fish. Res. Board Can. 32: 1041–1046.

MCLAREN, P. L., AND R. A. DAVIS. 1982. Winter distribution of arctic marine mammals in ice-covered waters of eastern North America. Report by LGL Ltd. to the Offshore Labrador Biological Program. 151 p. (Available from the Freshwater Institute, Winnipeg, Man.)

MCLAREN-MAREX. 1979a. Report on aerial surveys of marine mammals and birds in southern Davis Strait and eastern Hudson Strait in March, 1978. Report for Esso Resources Canada Ltd., Aquitaine Co. of Canada, and Canada Cities Services Ltd., Arctic Petroleum Association. Project No. 146, January. xxii + 36 p. + figures and tables. (Available from the Freshwater Institute, Winnipeg, Man.)

1979b. Report on aerial surveys of birds and marine mammals in the southern Davis Strait between April and December, 1978. Report for Esso Resources Canada Ltd., Aquitaine Co. of Canada, and Canada Cities Services Ltd., Arctic Petroleum Association. Project No. 146. February. xxii + 36 p. + figures and tables. (Available from the Freshwater Institute, Winnipeg, Man.)

MITCHELL, E., AND R. R. REEVES. 1981. Catch history and cumulative estimates of initial population size of cetaceans in the eastern Canadian Arctic. Rep. Int. Whaling Comm. 31: 645–682.

NORTON, P., AND L. A. HARWOOD. 1985. White whale use of the southeastern Beaufort Sea, July–September 1984. Can. Tech. Rep. Fish. Aquat. Sci. 1401: v + 46 p.

SMITH, T. G., and M. O. HAMMILL. 1986. Population estimates of white whale, *Delphinapterus leucas*, in James Bay, eastern Hudson Bay, and Ungava Bay. Can. J. Fish. Aquat. Sci. 43: 1982–1987.

SMITH, T. G., M. O. HAMMILL, D. J. BURRAGE, AND G. A. SLENO. 1985. Distribution and abundance of belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, in the Canadian high Arctic. Can. J. Fish. Aquat. Sci. 42: 676–684.

SOPER, J. D. 1944. The mammals of southern Baffin Island, Northwest Territories, Canada. J. Mammal. 25(3): 221–254.

STIRLING, I., AND H. CLEATOR. 1981. Polynyas in the Canadian Arctic. Can. Wildl. Serv. Occas. Pap. 45: 1–73.

STRONG, J. T. 1988. Status of the narwhal, *Monodon monoceros*, in Canada. Can. Field-Nat. 102: 391–398.

SUTTON, G. M., AND W. J. HAMILTON. 1932. The Mammals of Southampton Island. Part II. Section 1. p. 3–110. *In* The Exploration of Southampton island, Hudson Bay. Mem. Carnegie Mus. 12.

Mysis relicta Assimilation of Hexachlorobiphenyl from Sediments[1]

J. Val Klump

Center for Great Lakes Studies, Department of Biological Sciences and Department of Geosciences, University of Wisconsin-Milwaukee, 600 East Greenfield Avenue, Milwaukee, WI 53204, USA

and Jerry L. Kaster and Michael E. Sierszen[2]

Center for Great Lakes Studies, Department of Biological Sciences, University of Wisconsin-Milwaukee, 600 East Greenfield Avenue, Milwaukee, WI 53204, USA

Klump, J. V., J. L. Kaster, and M. E. Sierszen. 1991. *Mysis relicta* assimilation of hexachlorobiphenyl from sediments. Can. J. Fish. Aquat. Sci. 48: 284–289.

Assimilation and retention of a PCB congener, 2,4,5,2′,4′,5′-hexachlorobiphenyl (HCBP), by the oppossum shrimp, *Mysis relicta*, was followed during sediment ingestion and defecation using a ^{14}C label. Uptake was rapid and essentially linear, with mysids reaching a [^{14}C]HCBP specific activity equivalent to that in the labeled sediment within 2 wk. Assimilation efficiencies calculated from the relative depletion of the label in the fecal material averaged 53% (±7%). Sediment ingestion rates calculated from the activity required to supply the label retained were approximately 1 mg·mysid^{-1}·d^{-1}, in agreement with published estimates. The distribution of HCBP as a function of particle size indicates that particle-size selective feeding by detritivores can have a significant effect on calculated assimilation efficiencies. Given the vertical migration behavior of *M. relicta*, sediment ingestion is a potentially important pathway for the reintroduction of sediment-associated contaminants into the pelagic environment of deep lakes.

L'assimilation et la rétention d'un congénère des BPC, le 2,4,5,2′,4′,5′-hexachlorobiphényle (HCBP), ont été suivies au moyen du marqueur ^{14}C chez le mysis *Mysis relicta* durant l'ingestion de sédiments et la défécation. L'absorption était rapide et essentiellement linéaire, et en deux semaines, les mysis ont atteint un taux d'activité spécifique de [^{14}C]HCBP équivalente à celle des sédiments marqués. Les pourcentages d'assimilation calculés suivant la réduction relative de la quantité de ^{14}C dans les fèces étaient de 53 % (±7 %) Les taux d'ingestion de sédiments calculés suivant la quantité de sédiments à ingérer pour rendre compte de la quantité de ^{14}C assimilé étaient d'environ 1 mg·mysis^{-1}·j^{-1}, résultat corroborant les estimations déjà publiées. La distribution du HCBP en fonction de la taille des particules révèle que l'ingestion sélective des particules selon leur grosseur par les détritivores peut faire varier de façon importante les pourcentages d'assimilation calculés. Comme *M. relicta* effectue des migrations verticales, l'ingestion des sédiments peut s'avérer une voie importante de réintroduction dans la zone pélagique des lacs profonds des contaminants liés aux sédiments.

Received December 21, 1989
Accepted August 10, 1990
(JA419)

Reçu le 21 décembre 1989
Accepté le 10 août 1990

High sediment–water distribution coefficients (K_D) for many contaminants entering the aquatic environment result in efficient scavenging of these contaminants from solution by particles. When combined with active sedimentation processes, this physiochemical behavior leads to the relatively rapid movement of these contaminants into the benthic system, a process which has been shown to control the concentration of such pollutants in the waters of relatively closed basins such as the upper Great Lakes (Edgington 1981). For contaminants which are persistent, i.e. nonreactive biologically or chemically, their ultimate sink in closed basins is burial within the sediments.

Benthic macrofauna, however, play a key role in altering burial in sediments by actively mixing sediment accumulations equivalent to years or decades of input (Fisher et al. 1980). For example, the biologically mixed layer in Lake Michigan is on the order of 2 to 4 cm, which at an average linear sedimentation rate of 1 mm·yr^{-1} represents 20 to 40 yr of accumulated input. Many benthic organisms mix sediment as a result of feeding by ingestion of organic-rich debris falling out from the pelagic system above. In the profundal regions of the Great Lakes, the dominant benthic fauna are oligochaete worms, amphipods, and mysids (Cook and Johnson 1974; Nalepa 1989). Of these, all but the mysid, *Mysis relicta*, are essentially confined to the benthic environment. *Mysis*, however, is generally thought of as predominantly a pelagic zooplankter, well known for its diel migrations, and a major component of the pelagic food web as an omnivore (Cooper and Goldman 1980; Lasenby and Langford 1973; Grossnickle and Morgan 1979) and a forage component of fishes (Janssen 1978; Schneberger 1936; Van Oosten and Deason 1937; Wells and Beeton 1963). Moreover, Anderson and Smith (1971) reported that nearly every variety of fish occupying the deeper parts of Lake Superior feed on *M. relicta*. Mysids, however, also spend a significant amount of time in the benthic system, particularly during the day, where it has been suggested they actively feed on sediments (Mozley and Howmiller 1977; McWilliams 1970; Tattersall and Tattersall 1951). Recent observations include direct counts via manned submersibles and remotely operated camera surveys (Pachal et al. 1989). In our experience, sediment samples retrieved

[1]Contribution No. 344 of the Center for Great Lakes Studies.

[2]Current address: U.S. EPA Environmental Research Laboratory, Duluth, MN 55804, USA.

from the depositional environments of Lake Michigan during the day contain mysids in densities up to several hundreds per square metre in keeping with reported abundances (Morgan and Beeton 1978; Morgan 1981; McWilliams 1970).

Assimilation of contaminants by detritivores from ingested sediment is a potentially important pathway for maintaining contaminants within the biologically active zone of the ecosystem and delaying their ultimate burial. For mysids, assimilation from sediments has the added dimension of providing an extensive vertical transport pathway back into the pelagic food web as a result of diel migration patterns. It has been shown, for example, that mysids feeding on sediments ingest severalfold more zinc and cadmium than mysids feeding on zooplankton and that by upward migration the mysid population increases the amount of these metals in the water column at night (Van Duyn-Henderson and Lasenby 1986). Vertical downward migration during the day and fecal pellet fallout transport an equivalent amount of zinc and cadmium in the opposite direction. The net effect is an active recycling throughout the water column and a significant coupling between benthic and pelagic systems. These processes would apply to most sediment-associated constituents.

The extent to which such a mechanism prolongs the residence time of contaminants in the ecosystem depends in large part on the efficiency with which these contaminants are extracted from sediments and concentrated in the mobile vector, the organism. Some contaminants are essentially inert in this process, passing through the organism without being assimilated. This has led to the use of some elements (e.g. trivalent chromium, certain radionuclides, or biogenic silica) as conservative tracers for measuring feeding and defecation (Klump et al. 1987, and others cited therein). For some contaminants, particularly lipophilic organic compounds like PCBs, PAHs, and certain pesticides, assimilation across the gut wall during feeding results in an increase in the body burden of the contaminant above the concentration in the food (Eadie et al. 1982). Assimilation, defined as "absorption of material from the gut" (Johannes and Satomi 1967), therefore, serves to actively remove contaminants from the sediment burial pathway and reintroduce them into the biological compartment at a rate greater than would result from simple sediment mixing and transport processes. Oligochaete worms (Klump et al. 1987; Oliver 1984) and amphipods (Landrum 1982, 1989; Landrum and Scavia 1983) from the Great Lakes have been shown to concentrate organic contaminants from sediments. Hence, both the physical and biogeochemical dynamics of the benthic boundary layer, or nephaloid layer as it is often referred to, play an important role in contaminant fate in the Great Lakes. This paper reports *M. relicta* assimilation of a model PCB, 2,4,5,2′,4′,5′-hexachlorobiphenyl (HCBP), from ingested sediment using a radiotracer technique.

Materials and Methods

Assimilation Studies

Sediments used in this study were collected from a deepwater (115 m) depositional environment in Lake Michigan (43°09′54″N, 87°43′54″W). Numerous studies at this station have been conducted by Center for Great Lakes Studies personnel over the last two decades, including work on mysids. Sediments were collected using repeated casts of a gravity corer 12.7 cm (5 in.) in diameter from which only the upper 3 to 4 cm of sediment was saved. These fine-grained surficial sediments have an organic carbon content of ~2.8 to 3.6%, contain less than 10% of their particles in the greater than 63 μm fraction, and consist predominantly of particles less than 20 μm (Fitzgerald 1989; Klump et al. 1987).

Mysids, oligochaete worms, and amphipods actively feed on and burrow in the upper few centimetres of these sediments maintaining well-mixed conditions within this surface layer. Prior to introduction of the radiotracer the sediments were sieved using lake water through a 63-μm screen to remove macrofauna and trace amounts of fine sand and cinders (the latter occur frequently at this station, presumably as a result of the historical presence of coal-fueled shipping).

All glassware, stainless steel spatulas, etc., used in PCB tracer studies were cleaned according to the following protocol: scrubbed with 7× detergent and hot water, rinsed with double-distilled water, rinsed with 95% ethanol, allowed to dry, rinsed with pesticide analytical grade hexane, and combusted at 300 to 350°C for several hours. Sediments were labeled with UL-ring [^{14}C]HCBP (Pathfinder Laboratories) (specific activity = 14.06 μCi·μmol^{-1}) (1 μCi = 37 kBq). The crystalline HCBP tracer was first dissolved into a few millilitres of hexane. Hexane containing 50 to 100 μCi of [^{14}C]HCBP was coated on the inside of a 1000-mL round-bottom flask and then evaporated in an air stream leaving the HCBP adsorbed to the flask wall. Two hundred to 300 g of wet sediment was added to the flask which was then placed on a shaker for 2 d. The sediment slurry was then washed into a glass bottle and centrifuged at 3500 rpm for 10 min. The supernatant was decanted. The sediment pellet was transferred to a glass container using a small amount of lake water and stored at 4°C. In this manner, we obtained sediment activity of ~3600 dpm·mg^{-1}, equivalent to ~0.11 nmol [^{14}C]HCBP·mg sediment^{-1}. All weights reported refer to dry weights (at 65°C) unless otherwise specified.

Feeding chambers consisted of 20-cm-diameter glass aquaria containing 1.5 L of filtered 4°C lake water. A glass petri dish containing 1 cm of the labeled sediment was placed on the bottom. Mysids were then added to the aquarium which was kept in the dark at 4°C in an environmental chamber. Sediments from the dish were sampled periodically using a glass pasteur pipet. Fecal pellets deposited by the mysids while swimming in the overlying water were easily recognized and collected from the floor of the aquarium. Mysids were captured using a large-diameter glass pipet.

The total activity in the organism consists of both assimilated ^{14}C within the animal and unassimilated ^{14}C adsorbed to the sediments in the gut. In order to determine actual uptake, upon collection, mysids were placed immediately in a gut-clearing chamber which consisted of a 10-cm-diameter glass cylinder to which a glass funnel had been attached at the bottom. Silicone tubing was slipped over the end of the funnel stem and closed with a hose clamp. The neck of the funnel was partially blocked by placing a 3-cm piece of glass tubing bent at a 45° angle in the funnel. This allowed fecal pellets to drop into the neck of the funnel while it excluded the mysids and prevented reingestion of excreted material. Since *Mysis* will not quickly clear its gut except when actively feeding, the apparatus was first filled with 4°C lake water and a food source of *Fragilaria crotonensis* (a diatom alga isolated from Lake Michigan and batch cultured in modified Guillard's WC media (Guillard 1975) at 15°C under constant illumination). Complete clearing of sediments from the gut was easily observed in both the gut and fecal pellets by the distinct color change from black

sediment to light brown algae. This occurred rapidly, within 15 min to 1 h.

Both sediments and fecal pellets were dried to a constant weight at 65°C and weighed to the nearest 0.01 mg on tared 25-mm polycarbonate filter membranes. Individual mysids were dried at 65°C and then digested in 1 mL of NCS organic tissue solubilizer (Amersham Inc.) in a 20-mL glass scintillation vial using an ultrasonic bath followed by neutralization with glacial acetic acid. No loss of activity was observed in variable weights of fecal pellets or sediments dried for variable times up to several days. The potential loss of activity from mysids was never tested, but in similar experiments with tubificid worms, loss of HCBP was not observed during drying. Fecal pellets collected after each time interval were combined to facilitate accuracy in weighing. Both sediments and solubilized animals were then extracted in 15 mL of Aquasol for a minimum of 48 h. Sufficient water was then added to form a gel whereby the sediments were held in suspension. This significantly increased the relative counting efficiency for sediments and fecal deposits. ^{14}C activities were determined on a Beckman model 355 liquid scintillation counter using a standard ^{14}C window. Quench was determined using an external standard (^{137}Cs) channel ratio method and all counts were corrected to dpm.

[^{14}C]HCBP Adsorption Studies

In a separate set of experiments the distribution of HCBP on sediments as a function of particle size was determined. The procedure used was similar to that of diToro and Horzempa (1982) and consisted of equilibrating lake sediments with water containing [^{14}C]HCBP. Lake sediment was separated into four size classes using a centrifugal settling method (Jackson 1979). Each size class was brought into equilibrium with the [^{14}C]HCBP added to a particle–water slurry in a 35-mL Corex glass centrifuge tube. After equilibration, particles were separated from solution by centrifugation in a Sorval SS-3 at 12 000 rpm for ~20 min. The distribution of the tracer was determined from weighed aliquots of sediment particles and known volumes of 0.2- or 0.45-μm filtered supernatant.

Results and Discussion

Mysid Assimilation of [^{14}C]HCBP

Soon after introduction of the mysids into the experimental aquarium they began to actively burrow in the labeled sediments. The ^{14}C activity in the sediments, fecal pellets, and organisms was thereafter monitored over the course of 13 d (Table 1). Fecal pellet production was sparse, and although the mysids were ingesting and defecating sediments, it did not appear as if they were actively feeding at a very high rate. This became more apparent when (in order to induce them to clear their guts of ingested sediment) the organisms were given an abundant supply of diatoms. They initiated feeding on the diatoms almost immediately and fed very actively, as indicated by rapid fecal pellet production. Dissimilation of HCBP during this period (15 to 60 min) was assumed to be negligible in keeping with the measured half-life of HCBP in *M. relicta* of 142 to 576 h (Frez 1988).

The body burden of [^{14}C]HCBP as measured in individual mysids increased relatively constantly during the course of the experiment without showing any signs of leveling off (Fig. 1). The average rate of uptake calculated from the amount of activity incorporated into the organisms over time was approximately 250 dpm·mg dry weight^{-1}·d^{-1}. At that rate the mysids attained a [^{14}C]HCBP specific activity equivalent to that of the sediments in approximately 2 wk.

TABLE 1. Time course distribution of [^{14}C]HCBP in sediments, mysids, and fecal pellets over 13 d, and the assimilation efficiency calculated from the sediment/fecal pellet depletion using equation 4 (mean ± 1 SD).

	[^{14}C]HCBP specific activity			
Elapsed time (d)	Sediment (dpm·mg^{-1})	Mysids (dpm·mg^{-1})	Fecal pellets (dpm·mg^{-1})	Assimilation efficiency (%)
0	3820 ± 270	—	1923	46
1		322 ± 114	1815	49
2	3500 ± 200	534 ± 110	1305	63
3		845 ± 93	1693	52
6		—	1560	56
8		1127 ± 373	1465	59
13	3470 ± 215	3612 ± 247	1997	44
Mean	3550 ± 280		1680 ± 250	53 ± 7

Assimilation from the sediment (as opposed to from solution) may be measured from the depletion of the tracer in egested sediment relative to ingested sediment and may be defined in the following way (Johannes and Satomi 1967):

$$(1)\quad A = \mathrm{I} - U = R + D$$

$$(2)\quad E = U + D$$

where A = assimilation rate, I = ingestion rate, U = rate of release of unassimilated material, D = dissimilation rate (release of previously assimilated material), R = retention rate, and E = egestion rate.

Assimilation is usually determined with radiotracers as the sum of the activity retained plus the activity lost via dissimilation and metabolism. The latter, in particular, can confound measurements when rapid metabolic turnover occurs, for example through rapid respiration to carbon dioxide. For a persistent, refractory organic compound, metabolism may generally be ignored. Furthermore, in order to measure net assimilation only, no distinction need be made between unassimilated material and dissimilation of previously assimilated tracer. The net assimilation efficiency (AE), defined as the rate of retention relative to the rate of ingestion, may be determined indirectly from tracer ingestion and egestion rates (Conover 1966):

$$(3)\quad \mathrm{AE} = R/I = (I - E)/I.$$

Assuming that for a detritivore feeding upon sediment, the rates of sediment ingestion and egestion are essentially equal, i.e. there is no significant change in the mass of sediments as they pass through the organism, assimilation efficiencies for the radiotracer may be calculated from the relative depletion of the tracer as it passes through the gut via the following expression (Conover 1966; Calow and Fletcher 1972):

$$(4)\quad \%\mathrm{AE} = 1 - \frac{[^{14}\mathrm{C\ dpm{\cdot}mg^{-1}}]\ \text{egesta}}{[^{14}\mathrm{C\ dpm{\cdot}mg^{-1}}]\ \text{sediment}} \times 100.$$

This assumption, low assimilation of sediment mass, was not directly tested. Lasenby and Langford (1973) did measure significant changes in the mass egested but only for mysids

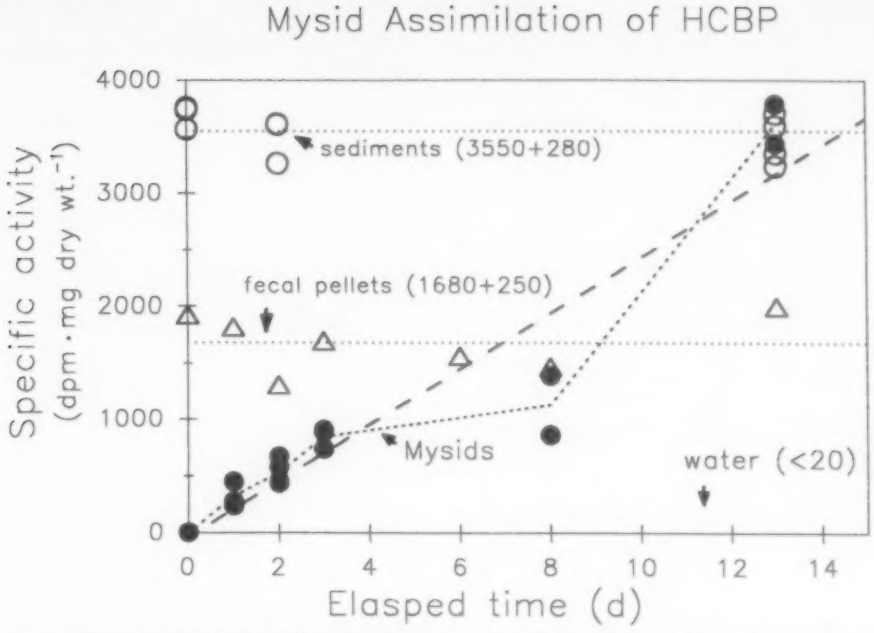


FIG. 1. Distrubition of [^{14}C]HCBP in sediments (open circles), fecal pellets (open triangles), and mysids (solid circles) during the course of mysid feeding on sediment. The linear regression to the mysid data yields an average retention rate of 245 dpm·mg mysid^{-1}·d^{-1} (broken line, $r^2 = 0.89$). ^{14}C activity in the overlying water was undetectable (<20 dpm·mL^{-1}).

TABLE 2. Adsorption of [^{14}C]HCBP on several size fractions of lake sediment.

Particle size range (μm)	Abundance in weight (%)	[^{14}C]HCBP specific activity		K_D (dpm·g sediment^{-1}/ (dpm·mL water^{-1})
		Water (dpm·mL^{-1})	Sediment (dpm·mg^{-1})	
63–20	9	—	—	—
20–5	50	223	428 ± 22	1.85×10^3
5–2	26	255	3975 ± 94	1.56×10^4
2–0.45	14	574	$1.226 \pm 0.04 \times 10^5$	2.14×10^5

feeding on *Daphnia* and other cladocerans which consisted of 70 to 80% organic matter. The organic carbon content of the sediments used here, and typical of Lake Michigan, is ~3% by weight, i.e. the "assimilatible fraction" is a relatively minor component of the sediment as a whole. For changes in mass to affect these calculations would require assimilation of a significant percentage of the >90% inorganic mineral matter found in these sediments.

The measured specific activity of the sediment available to the mysids did not vary significantly during the course of the experiment and averaged 3550 (±280 SD) dpm·mg^{-1}. Fecal pellets appeared to be significantly depleted in the HCBP label, however, and ranged from 1300 to 2000 dpm·mg^{-1} with an average of 1680 (±250 SD) dpm·mg^{-1}. Using equation 4, net assimilation efficiencies were calculated from the relative depletion of [^{14}C]HCBP in the fecal material and averaged 53% (±7%) (Table 1). This relatively high assimilation efficiency is apparently not unusual. Marine copepods have been observed to retain 60 to 70% of ingested DDT during feeding on contaminated phytoplankton (Harding et al. 1981). In similar studies with tubificid worms, we have observed assimilation efficiencies for HCBP ranging from ~15 to 40% in actively feeding animals (Klump et al. 1987).

One caveat worth noting, with regard to the calculation of assimilation efficiencies via the formalism of equation 4, is illustrated in Table 2. The distribution of many sediment-associated contaminants is not uniform. The dependence of particle size on distribution coefficients is related to the fact that many contaminants associate with sediments via surface interactions; hence, smaller particles with higher surface area to volume ratios contain higher concentrations of adsorbed species on a mass basis. Benthic detritivores are well known for being particle-size selective feeders (Hargrave 1970; Krezoski and Robbins 1985). Mysids, for example, have been observed to have a percentage of particles from 2.5 to 5 μm in their guts that is higher than that in the substrate, i.e. they select for this smaller particle size class, presumably because of higher organic content and microbial densities (Lasenby and Langford 1973). The impact of such behavior would be to alter the specific activity (or concentration) of HCBP in the ingested sediment, and consequently, change the assimilation required to bring HCBP activity to the level observed in the fecal pellets. In fact, at the K_D values given in Table 2 the specific activity or concentration in the sediment is controlled by the smallest size class measured, 2 to 0.45 μm. While only 14% of the

sediment mass, it contained 86% of the activity. The effect on assimilation depends entirely on the relative selectivity of the feeding and the assimilation efficiency for HCBP in each particle size class. Particle size dependent calculations require knowledge of both, but the potential magnitude of the effect can be illustrated from these data. For example, feeding which reverses the relative abundance of particles ingested from 50 and 26% in the 20- to 5-μm and 5- to 2-μm classes (as in the bulk sediment) to 26 and 50%, only increases the bulk sediment specific activity by ~10% and, in the calculation given above, would only increase the net bulk assimilation efficiency from an average of 53% to an average of 57%, a relatively insignificant change. A similar doubling of the percentage of particles ingested of the smallest size class (2 to 0.45 μm), however, would increase the required assimilation efficiency to nearly 75%. Here, in order to determine size-dependent partitioning, sediment size fractions were separated first and then labeled. The sediment for feeding experiments, on the other hand, was labeled in bulk. The possibility for differences in partitioning resulting from these two different procedures was unexplored, but the trend for increasing partition coefficients with decreasing particle size should remain significant for HCBP, as well as most hydrophobic contaminants. More specific information on the assimilation of bioconcentrated contaminants will, therefore, in many instances require particle size specific models of feeding (Klump et al. 1987).

Because mysids feed and defecate while burrowing in mud, direct measurements of ingestion or egestion rates are not possible. A rough estimate may be made, however, by calculating the mass of sediment required to be ingested at the assimilation efficiency measured in order to supply the retention observed directly as the increasing body burden with time, i.e.

$$(5) \quad I = R/(AE \cdot a)$$

where a is the specific activity of the sediment ingested (3550 (±280 SD) dpm·mg sediment^{-1}). At an average net retention rate of 250 dpm·mg mysid^{-1}·d^{-1} and average net assimilation efficiency of 53%, ingestion rates required were approximately 0.13 mg·mg mysid^{-1}·d^{-1}. The average dry weight of the mysids in this experiment was 7.42 (±2.70 SD) mg·individual^{-1}, giving an average sediment ingestion rate of 1 mg sediment·individual^{-1}·d^{-1}. In experiments with *Neomysis*, a similarly sized mysid, feeding on *Daphnia* Murtaugh (1984), measured egestion rates varying from 0.02 to 2 mm^3·individual^{-1}·d^{-1}, a similar rate, From the metal ingestion data of Van Duyn-Henderson and Lasenby (1986), *M. relicta* feeding rates are calculated to be on the order of ~0.3 mg sediment·mysid^{-1}·d^{-1}. Hence the assimilation efficiencies and retention rates measured for HCBP in this experiment are consistent with reported sediment ingestion rates for *M. relicta*. It must be pointed out that this formulation assumes that all of the HCBP assimilated was derived from ingested sediment whereas this may not entirely be the case. Accumulation of sediment associated contaminants in other Great Lakes benthos, specifically *Pontoporeia hoyi*, has been shown to occur both from ingested sediment and from interstitial water (Landrum 1989). The extent to which assimilation from solution occurred in these experiments cannot be determined. The sediment ingestion rates calculated represent maximums which would be lowered in direct proportion to the fraction of uptake that occurred from the water. Even given this uncertainty, calculated feeding rates are reasonable, i.e. the organism does not have to ingest unusual quantities of sediment to explain the observed uptake via the sediment assimilation pathway alone.

It is likely that mysids do not actively rely on sediments as a primary source of nutrition for growth, but rather seek sediments as a refuge or inhabit the benthic boundary layer as predators on deposit-feeders such as *Pontoporeia* (Parker 1980). However, mysids do burrow into and ingest sediments; this exposes them to high concentrations of certain contaminants. For those contaminants which are lipophilic in nature, efficient assimilation may result in their rapid removal from sediments and reentry into the pelagic food web via an organism which plays an important role in benthic–pelagic coupling mechanisms in deep lakes.

Acknowledgements

This work was supported by the U.S. National Oceanic and Atmospheric Administration (grant No. NA81RA00029) and the Center for Great Lakes Studies, University of Wisconsin-Milwaukee.

References

ANDERSON, E. D., AND L. L. SMITH, JR. 1971. A synoptic study of food habits of 30 fish species from western Lake Superior. Univ. Minn. Agric. Exp. Stn. Tech. Bull. 279: 199 p.

CALOW, P., AND C. R. FLETCHER. 1972. A new radiotracer technique involving ^{14}C and ^{51}Cr for estimating the assimilation efficiencies of aquatic primary consumers. Oecologia 9: 155–170.

CONOVER, R. J. 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11: 338–345.

COOK, D. C., AND M. G. JOHNSON. 1974. Benthic macroinvertebrates of the St. Lawrence Great Lakes. J. Fish. Res. Board Can. 31: 763–782.

COOPER, S. D., AND C. R. GOLDMAN. 1980. Opossum shrimp (*Mysis relicta*) predation on zooplankton. Can. J. Fish. Aquat. Sci. 37: 909–919.

DITORO, D. M., AND L. M. HORZEMPA. 1982. Reversible and resistant components of PCB adsorption–desorption isotherms. Environ. Sci. Technol. 16: 594–602.

EADIE, B. J., W. R. FAUST, P. F. LANDRUM, N. R. MOREHEAD, W. S. GARDNER, AND T. NALEPA. 1982. Bioconcentration of PAH by some benthic organisms in the Great Lakes, p. 437–449. *In* M. W. Cooke and A. J. Dennis [ed.] Polynuclear aromatic hydrocarbons: Seventh International Symposium on Formation, Metabolism and Measurement. Battelle Press, Columbus, OH.

EDGINGTON, D. N. 1981. A review of the persistence of long-lived radionuclides in the marine environment — sediment-water interactions, p. 67–91. *In* Impacts of radionuclide releases into the marine environment. IAEA-SM-248/148.

FISHER, J. B., W. J. LICK, P. L. MCCALL, AND J. A. ROBBINS. 1980. Vertical mixing of lake sediments by tubificid oligochaetes. J. Geophys. Res. 85: 3997–4006.

FITZGERALD, S. A. 1989. The biogeochemistry of amino acids in sediments from the Great Lakes. Ph.D. dissertation, The University of Wisconsin-Milwaukee, Milwaukee, WI. 250 p.

FREZ, W. A. 1988. The toxicokinetics of hydrophobic contaminants in the invertebrate *Mysis relicta*. Ph.D. dissertation, The University of Michigan, Ann Arbor, MI. 186 p.

GROSSNICKLE, N. E., AND M. D. MORGAN. 1979. Density estimates of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 36: 694–698.

GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 69–85. *In* W. L. Smith and M. H. Chanley [ed.] Culture of marine invertebrate animals. Plenum Publ. Co., New York, NY.

HARDING, G. C., W. P. VASS, AND K. F. DRINKWATER. 1981. Importance of feeding, direct uptake from seawater, and transfer from generation to generation in the accumulation of an organochlorine (p,p'-DDT) by the marine planktonic copepod *Calanus finmarchicus*. Can. J. Fish. Aquat. Sci. 38: 101–119.

HARGRAVE, B. T. 1970. The utilization of benthic microflora by *Hyallela azteca* (Amphipoda). J. Anim. Ecol. 39: 427–437.

JACKSON, M. L. 1979. Soil chemical analysis. 2nd ed. 11th printing. Published by the author, Madison, WI 53705.

JANSSEN, J. 1978. Feeding-behavior of the alewife *Alosa pseudoharengus* and the ciscoes *Coregonus hoyi* and *C. artedii*. J. Fish. Res. Board Can. 35: 249–253.

JOHANNES, R. E., AND M. SATOMI. 1967. Measuring organic matter retained by aquatic invertebrates. J. Fish. Res. Board Can. 24: 2467–2471.

KLUMP, J. V., J. R. KREZOSKI, M. E. SMITH, AND J. L. KASTER. 1987. Dual tracer studies of the assimilation of an organic contaminant from sediments by deposit feeding oligochaetes. Can. J. Fish. Aquat. Sci. 44: 1574–1583.

KREZOSKI, J. R., AND J. A. ROBBINS. 1985. Vertical distribution of feeding and particle-selective transport of ^{137}Cs in lake sediments by lumbriculid oligochaetes. J. Geophys. Res. 90: 11999–12006.

LANDRUM, P. F. 1982. Uptake, depuration and biotransformation of anthracene by the scud *Pontoporeia hoyi*. Chemosphere 11: 1049–1057.

1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. Environ. Sci. Technol. 23: 588–595.

LANDRUM, P. F., AND D. SCAVIA. 1983. Influence of sediment on anthracene uptake, depuration and biotransformation by the amphipod *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 40: 298–305.

LASENBY, D. C., AND R. R. LANGFORD. 1973. Feeding and assimilation of *Mysis relicta*. Limnol. Oceanogr. 15: 280–285.

MCWILLIAMS, P. S. 1970. Seasonal changes in abundance and reproduction in the opossum shrimp *Mysis relicta* Loven in Lake Michigan. M.S. thesis, University of Sydney, Sydney, Australia. 130 p.

MORGAN, M. D. 1981. Abundance, life history, and growth of introduced populations of the opossum shrimp (*Mysis relicta*) in subalpine California lakes. Can. J. Fish. Aquat. Sci. 38: 989–993.

MORGAN, M. D., AND A. M. BEETON. 1978. Life history and abundance of *Mysis relicta* in Lake Michigan. Can. J. Fish. Aquat. Sci. 35: 1165–1170.

MOZLEY, S. C., AND R. P. HOWMILLER. 1977. Environmental status of the Lake Michigan Region. Vol. 6. Zoobenthos of Lake Michigan. Argonne National Lab., Argonne, IL. 148 p.

MURTAUGH, P. A. 1984. Variable gut residence time: problems in inferring feeding rate from stomach fullness of a mysid crustacean. Can. J. Fish. Aquat. Sci. 41: 1287–1293.

NALEPA, T. F. 1989. Estimates of macroinvertebrate biomass in Lake Michigan. J. Great Lakes Res. 15: 437–443.

OLIVER, B. G. 1984. Uptake of chlorinated organics from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. 41: 878–883.

PACHAL, V., B. FARROW, D. HALL, AND B. COOPER. 1989. Midwater ecology of mysids in Lake Michigan. Abstr., 32nd Conf. on Great Lakes Res. p. 90.

PARKER, J. I. 1980. Predation by *Mysis relicta* on *Pontoporeia hoyi*: a food chain link of potential importance in the Great Lakes. J. Great Lakes Res. 6: 164–166.

SCHNEBERGER, E. 1936. The biological and economic importance of the smelt in Green Bay. Trans. Am. Fish. Soc. 66: 139–142.

TATTERSALL, W. M., AND O. S. TATTERSALL. 1951. The British Mysidacea. Ray Society, London. 460 p.

VAN DUYN-HENDERSON, J. A., AND D. C. LASENBY. 1986. Zinc and cadmium transport by the verically migrating opossum shrimp, *Mysis relicta*. Can. J. Fish. Aquat. Sci. 43: 1726–1732.

VAN OOSTEN, J., AND H. J. DEASON. 1937. The food of the lake trout *Cristivomer namaycush* and the lawyer *Lota maculosa* of Lake Michigan. Trans. Am. Fish. Soc. 67: 155–177.

WELLS, L., AND A. M. BEETON. 1963. Food of the bloater, *Coregonus hoyi*, in Lake Michigan. Trans. Am. Fish. Soc. 92: 245–255.

Range Detection of Targets using Hydroacoustics in the Laboratory

P. H. Patrick, B. Sim, and G. Hunt

Biological Research Section, Ontario Hydro Research Division, 800 Kipling Avenue, Toronto, Ont. M8Z 5S4, Canada

Patrick, P. H., B. Sim, and G. Hunt. 1991. Range detection of targets using hydroacoustics in the laboratory. Can. J. Fish. Aquat. Sci. 48: 290–295.

Investigations were conducted in the laboratory on the accuracy in terms of range resolution of a commercial sonar system (420 kHz) in detecting underwater targets. Accurate range resolution of fish is necessary for reliable estimates of numbers and for detecting the presence and behaviour of fish near the bottom. Results indicated that the existing sounder was limited in target separation below 25 cm at a transmitted pulse of 0.1 ms under laboratory conditions. Modifications to equipment resulted in an improved range resolution (to approximately 10 cm) of targets in both vertical and horizontal tank experiments.

Les auteurs ont effectué des travaux expérimentaux sur la précision, en terme de pouvoir séparateur en portée, d'un sonar commercial (420 kHz) pour la détection de cibles sous-marines. Un pouvoir séparateur en portéeprécis des poissons est nécessaire pour obtenir des estimations fiables du nombre et pour déceler la présence et le comportement des poissons près du fond. Les résultats obtenus ont révélé que le sondeur actual ne pouvait distinguer des cibles à moins de 25 cm de distance à une impulsion transmise de 0,1 ms dans des conditions expérimentales. Des modifications de l'équipement ont permis d'obtenir un meilleur pouvoir séparateur en portée, verticale et horizontale, (jusqu'à environ 10 cm) des cibles dans des bassins expérimentaux.

Received January 10, 1990
Accepted August 10, 1990
(JA435)

Reçu le 10 janvier 1990
Accepté le 10 août 1990

Recently, there has been considerable interest in the use of hydroacoustics for obtaining fisheries data (Rose and Leggett 1988; Thorne 1988). Advancements have been made in the areas of developing real time data collection and analysis systems (Dickie et al. 1983; Brooks and Torkelson 1987; Mitchell et al. 1987). Another significant contribution has been the development of the dual beam (Traynor and Ehrenberg 1979) and split beam (Foote and Traynor 1988) systems which allow for off-axis correction of target strengths. Still, much work has to done before hydroacoustics can replace to a large extent current netting practices in the collection of fisheries data.

At present, there is some concern over the ability of commercial sonar systems to successfully resolve targets in the water column. Range resolution is the ability of sonar to distinguish two objects, each having a different range to the sonar. It is important because estimates of single fishes and schools are based on accurate counting of targets. Furthermore, assessment of fish and their behaviour near the bottom of a lake or river requires accurate range resolution. Counting may also be the preferred method over integration to estimate biomass of the species, especially if fish densities are low. We determined the range resolution of targets using a commercial sonar system and modified the system to improve its resolution.

Materials and Methods

Hydroacoustic Equipment

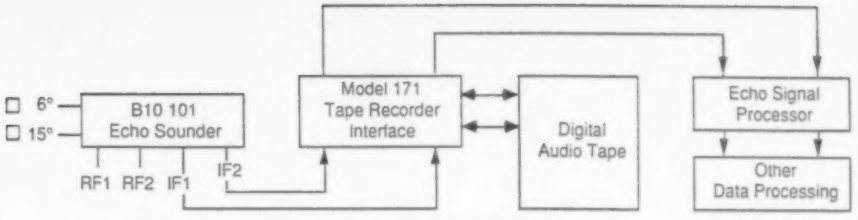
Acoustic sampling was conducted using the following equipment (Fig. 1); 420-kHz dual beam transducer (Biosonics, $6 \times 15°$), sounder (Biosonics 101), transducer rotator (Biosonics RC), tape recorder interface (Biosonics 171), digital audio tape recorder (Sony DTC 1000), frequency modulation recorder (Racal Sore 7), oscilloscope (Phillips PM 3302), plotter (Hewlett-Packard 7046 A), dual beam processor (Biosonics ESP 181), PC computer (AT 238 40 MB), and printer (Epson FX). The DAT was capable of recording only AC signals whereby the recorder interface was used to develop a full wave detected envelope at playback. Since it was only a two-channel recorder, only the existing data (6 and 15°) could be recorded on this instrument. The multichannel Racal was used for recording of the signals using both the WSDM modification and the existing sounder. All data were collected simultaneously using both the DAT (existing sounder) and FM Racal (existing sounder, WSDM) recording systems. Results from the existing sounder for both the DAT and FM recorder were comparable.

The Biosonics ESP is not recommended (Brooks and Torkelson 1987) for use at pulse widths less than 0.2 ms because the sampling rate of the processor is limited (25 kHz). However, by recording the signals at approximately 75 cm/s (30 in./s) with the FM Racal, the recorded bandwidth would be approximately 20 kHz (adequate for the 0.1-ms pulse, Clay and Medwin 1977). When the signals are played back at 18.7 cm/s, a 4:1 time expansion would result in the 0.1-ms pulse being 0.4 ms, allowing the signal to be processed by the ESP.

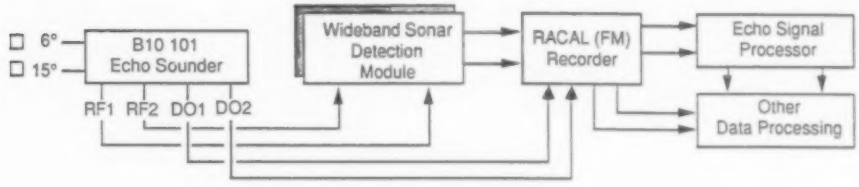
Comparisons in data collections between the existing sounder and modified one (WSDM) were made at two different transmitted pulse widths (0.1 and 0.5 ms). These pulse widths theoretically corresponded to range resolutions of approximately 7.5 and 37.5 cm, respectively (Mitson 1983).

Preliminary Inspection of Sounder

A preliminary inspection of the sounder indicated that filtering of the received signal occurred prior to the intermediate



A. EXISTING SYSTEM



B. MODIFIED SYSTEM

FIG. 1. Block diagrams showing data collection systems for both modified and existing echo sounders.

frequency and detected output (Patrick 1989). This filtering resulted in a rounding off of the pulses and a loss of information, especially at the lower pulse widths (0.1 ms). Although a 10-kHz bandwidth is adequate to pass a 0.1-ms pulse (Clay and Medwin 1977), we believe a higher bandwidth (up to 20 kHz) is necessary to provide more detail of the pulse necessary for improved range resolution. We felt that valuable information in the reflected signal necessary for optimizing range resolution would be lost because of this filtering. For this reason, a wideband sonar detection module (WSDM) was developed which used the radio frequency (RF) output of the sounder and allowed up to 30-kHz bandwidth information to be recorded.

Experimental Facilities

Acoustical sampling involved placing the transducers both horizontally and vertically in two separate tanks. The horizontal tank measured approximately 15.5 m in length and width and was 1.5 m deep. The tank was made semianechoic in the audible range by constructing a perimeter wall of 25-cm concrete blocks (with the opening facing the water) approximately 1.0 m inside the tank. The dual beam transducer was placed at one end of the tank, and information on targets was obtained within 3–9 m from the transducer.

The vertical tank measured 4.4 m in depth and had a diameter of 3.1 m. The dual beam transducer was placed just below the surface. Information on targets was obtained from approximately 2.1–4.2 m from the transducer.

Range Resolution Experiments in Water Column

For experiments conducted in the horizontal tank, three ping-pong balls, each approximately 10–15 cm apart, were placed 3, 5, 8, and 9 m from the transducer (i.e. a total of 12 targets). The existing and modified sounder were compared in their ability to detect all 12 targets based on oscilloscope tracings and target strength analysis involving single target detection using Biosonics ESP (Dawson et al. 1989). The presence of single targets, if they occur, can be isolated using voltage and pulse width criteria. The pulse width and voltage criteria for a 0.5-ms pulse was 0.4 and 0.7 ms and 0.16 V, respectively. For a 0.1-ms pulse (stretched to 0.4 ms), the pulse width criteria were 0.3 and 0.6 ms. The voltage criteria was 0.16 V.

The vertical tank experiment concentrated on determining the resolution of a ping-pong ball at various distances from the tank bottom (15, 30, 45, 60, and 90 cm for a 0.1-ms pulse). The tank bottom was smooth (concrete) and was approximately 4.1 m from the transducer. Range resolution comparisons were made between the existing and modified sounder.

Results and Discussion

Horizontal Experiments

A comparison of the range resolution between the existing and the WSDM systems for ping-pong balls placed at different distances from the transducer indicated a greater resolution for the modified unit (Fig. 2). This was especially evident for the 0.1-ms transmitted pulse results. Most balls could be distinguished from one another or counted (nine balls) using the modified system. This resolution was not evident with the unmodified unit, as it was difficult to count the targets from the oscilloscope tracings. Results of the ESP analysis on the 0.1-ms data supported the above results. Nine of 12 ping-pong balls could be isolated as individual targets using the ESP. Individual ping-pong balls could not be separated by the ESP using data collected with the existing sounder. The pulse width data were not accepted. For both the existing and modified sounder data sets, range resolution became distorted approximately 9 m from the transducer. The distortion was most likely related to interferences from other targets closer to the transducer (3- to 8-m distances). It is also possible that these targets were placed too close together (less than 10 cm apart). As expected, resolution using a 0.5-ms pulse was limited for both the existing and modified sounder (theoretical resolution is approximately 37.5 cm, Mitson 1983).

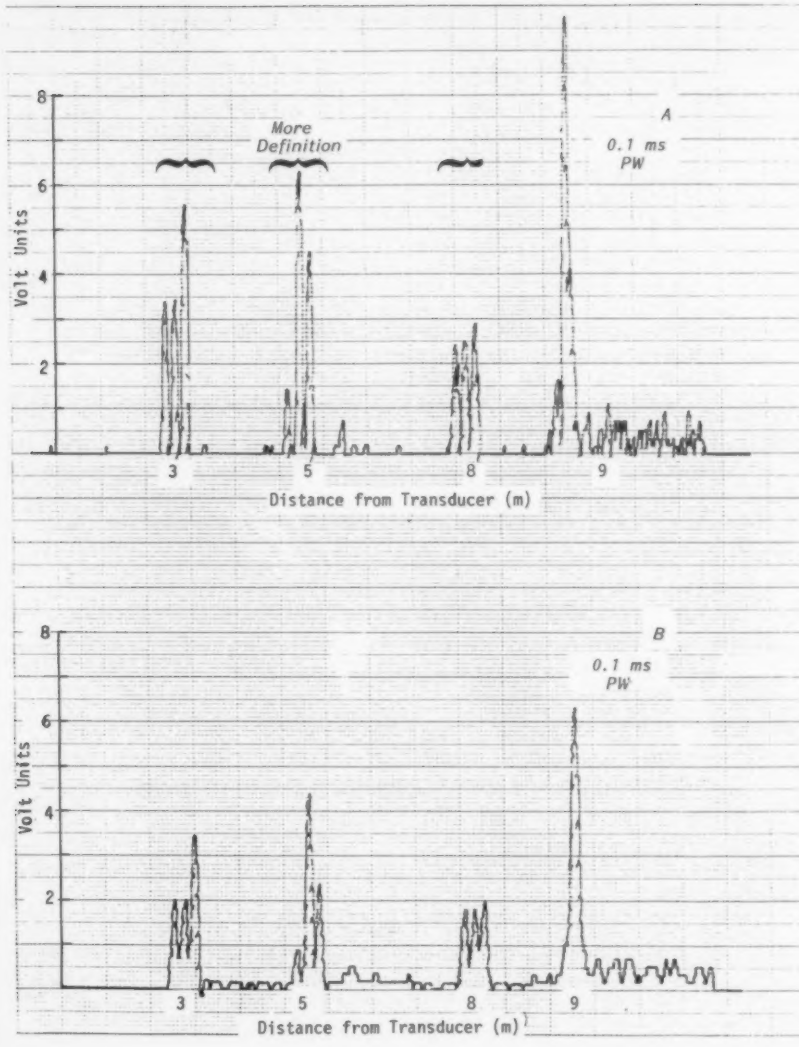
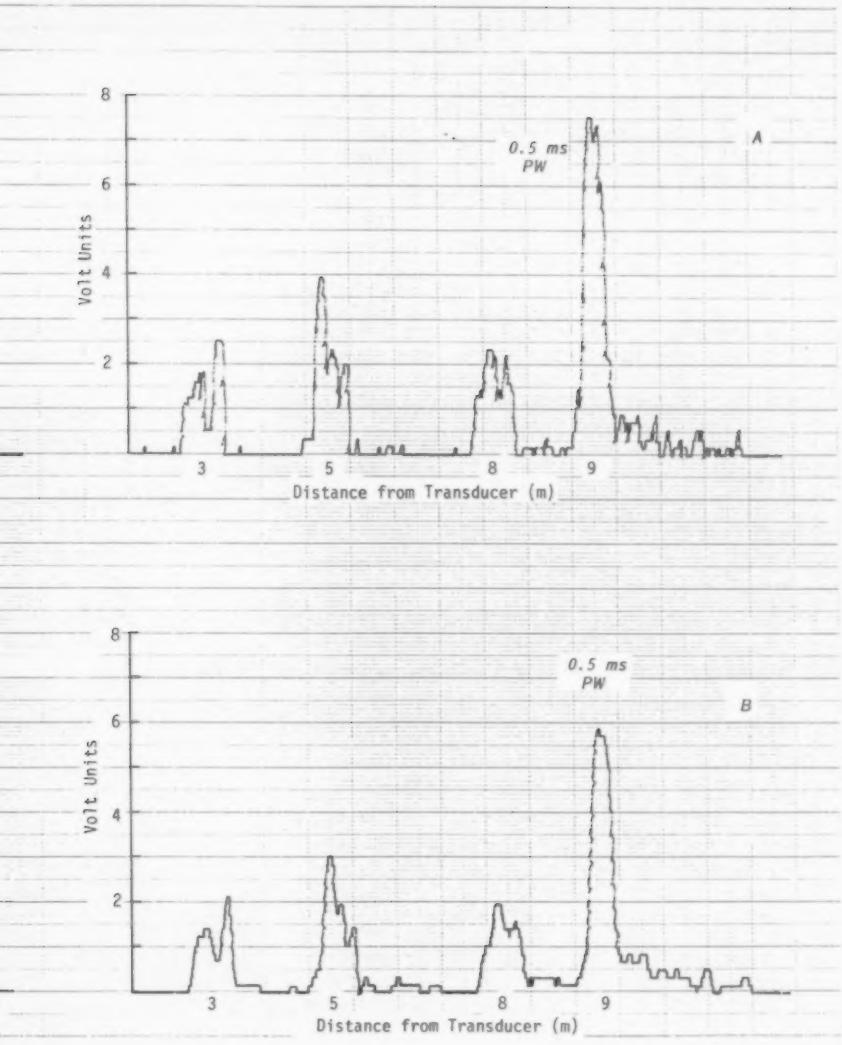


FIG. 2. Comparison of target (3) separation at various distances fro



nces from the transducer for both modified (A) and existing (B) sounders.

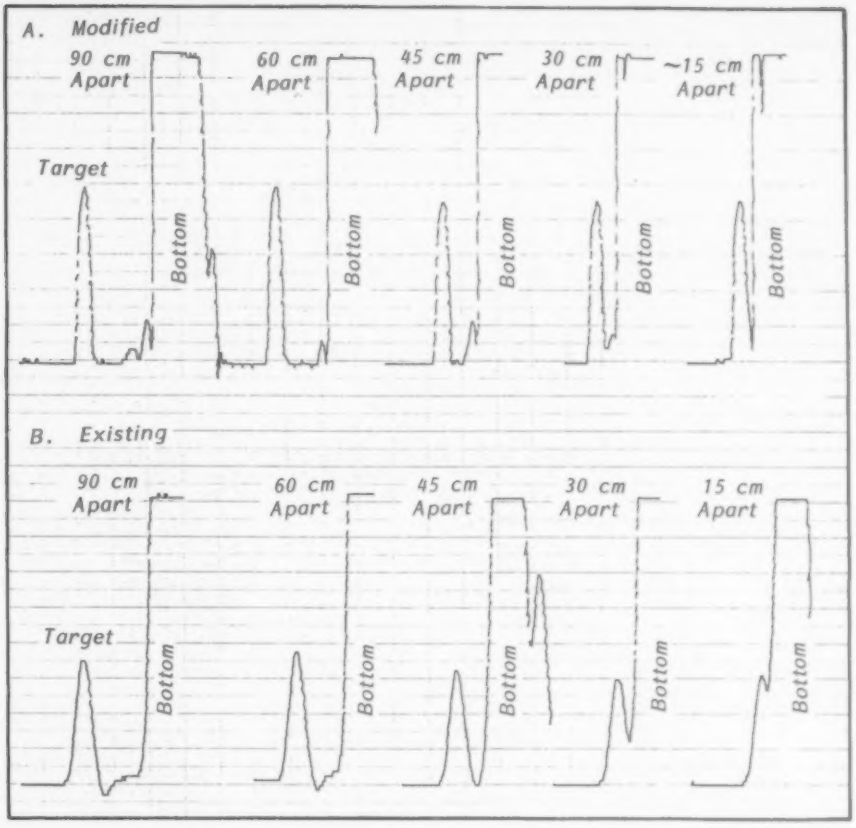


FIG. 3. Comparison of target separation (0.1-ms pulse) at various distances from the bottom for both modified (A) and existing (B) sounders.

Vertical Tank Experiments

Results from the vertical tank showed a similar trend to that found in the horizontal tank (Fig. 3 and 4). Data collected using the modified system gave better range resolution than the existing sounder. Range resolution was improved considerably with the WSDM modification. With the existing sounder, separation of a ping-pong ball from the tank bottom was difficult at a distance less than 30 cm for a 0.1-ms pulse. Results with the WSDM indicated an improved target separation from the bottom (to at least 15 cm). Further experiments in the vertical tanks (not shown in Fig. 3) indicated a resolution of approximately 10 cm.

Interpretive Analysis

Range resolution of targets is important because estimates of fish numbers are based on accurate counting of single targets. Other factors such as the transducer beam width and the distance between the school and the transducer are also important factors in determining fish abundance. Many species of fish school at high densities and detection of individual targets is problematic (Dickie et al. 1983). The resolution required to count fish is, of course, dependent on the behaviour of the species and its size. Nearest neighbour distance (NND) has often been used to describe the distance between individuals in a school and could approximate required range resolution for echo counting of individual fish. Pitcher and Partridge (1979) have shown that NND averaged approximately one body length based on laboratory results. Rose and Leggett (1988) have shown that NND can exceed three body lengths based on field data. Still, others have suggested that NND can vary from 0.3 to 1.8 body length for the same species (Glass et al. 1986).

Our results indicated that the commercial sounder may be limited in its ability to separate individuals in close proximity. Preliminary laboratory results indicated that the sounder had difficulty separating targets less than 25 cm apart approximately 3 m from the transducer. The modified sounder improved the resolution to approximately 10 cm using a 0.1-ms pulse resulting in greater range detection for counting individual fish and also for estimating target strengths of individuals in a school. Still, our results were based on a 420-kHz transducer, and the present resolution may deteriorate for lower frequency transducers. There is also a theoretical distance at which the resolution of a specific pulse width deteriorates. The sonar equation could be used to calculate this distance and

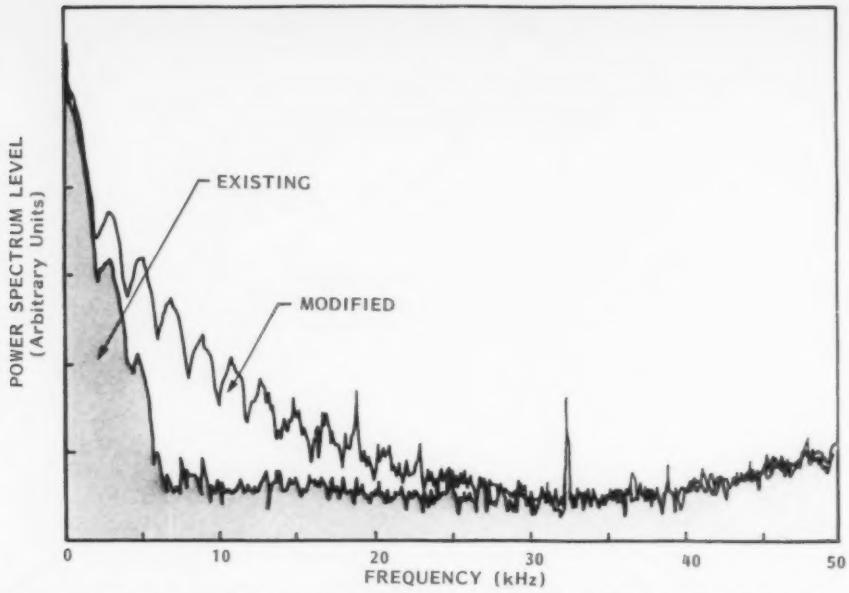


FIG. 4. Typical power spectrum of a ping-pong ball for existing and modified sounders (0.5-ms transmitted pulse).

would involve analyzing data on propagation losses, reverberation levels, and noise levels.

Although the WSDM modification improved the resolution of the existing sounder, further improvements can still occur. These improvements can focus on shortening the transmitted pulse lower than 0.1 ms (to at least 0.075 ms based on the bandwidth of the WSDM). The trade-offs in improved range resolution that will most likely have to be considered are a loss in transmission power and a further increase in background noise attributed to an increase in bandwidth. The former is not considered a serious problem, since the distances involved in fisheries research are generally less than 100 m. Recent field trails using this system in Lake Ontario have indicated no significant loss in signal-to-noise over a 50-m distance.

Another potential advantage of the WSDM modification is for easier classification of individual signals. If fish identification is based solely on the reflected signals of individual targets (as opposed to school discrimination, e.g. Rose and Leggett 1988), then detailed information in the reflected signal would presumably be a requirement for classification. Preliminary results of the reflected signals of a ping-pong ball indicated that more information (fourier components) was available using the modified sounder than the existing one (Fig. 4). Similar results were obtained observing the reflected signals of several fish species in the laboratory (Patrick 1989). At present, several methods can be considered to resolve differences in the reflected signals of various underwater targets and these include filtering techniques (e.g. Deuser et al. 1979) and pattern recognition systems such as neural networking (Ramani et al. 1989). The latter is currently being investigated for echo interpretation.

Acknowledgments

Mike MacKay wrote the MACRO for separating targets and estimating target strengths and analyzed most of the data using the Biosonics ESP. We would like to thank Biosonics for their help during this work. We also thank the two reviewers for their comments.

References

BROOKS, T. J., AND T. C. TORKELSON. 1987. Applications of new technology to fisheries hydroacoustics and analysis. Int. Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987.

BURCZYNSKI, J. J., AND R L. JOHNSON. 1986. Application survey techniques to limnetic populations of juvenile sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 43: 1776–1788.

CLAY, C. S., AND H. MEDWIN. 1977. Acoustical oceanography: principles and applications. Wiley, New York, NY.

DAWSON, J. J., T. J BROOKS, AND E. S. KUEHL. 1989. An innovative acoustic signal processor for fisheries science. Proc. Inst. Acoust. 3: 131–140.

DICKIE, L. M., R. G. DOWD, AND P. R. BOUDREAU. 1983. An echo-counting and logging system (ECOLOG) for demersal fish size distributions and densities. Can. J. Fish. Aquat. Sci. 40: 487–498.

DEUSER, L. M., D. MIDDLETON, T. D. PLEMONS, AND J. K. VAUGHAN. 1979. On the classification of underwater acoustic signals. II. Experimental applications involving fish. J. Acoust. Soc. Am. 65: 444–455.

FOOTE, K. G., AND J. J. TRAYNOR. 1988. Comparison of walleye pollock target strength estimates determined from in situ measurements and calculations based on swimbladder form. J. Acoust. Soc. Am. 73: 1932–1940.

GLASS, C. W., C. S. WARDLE, AND W. R. MOSJSIEWICZ. 1986. A light intensity threshold for schooling in the Atlantic mackerel, *Scomber scombrus*. J. Fish Biol. 29: 71–81.

MITCHELL, R. S., A. STEPNOWSKI, J. DROBNIK, AND D. R. GREEN. 1987. Real time signal processing in a dual beam sonar system for fish stock assessment. Int. Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987.

MITSON, R. B. [ED.] 1983. Fisheries sonar. Fishing News Book Ltd., Surrey, England.

MULLIGAN, T. J., R. KIESER, AND T. GJERNES. 1987. A model for comparing fish density estimates from seine catches and from echo integration. Can. J. Fish. Aquat. Sci. 44: 1024–1030.

PATRICK, P. H. 1989. Remote sensing using hydroacoustics. Ont. Hydro Res. Div. Rep. No. 89-21-H, April 13, 1989.

PITCHER, T. J., AND B. L. PARTRIDGE. 1979. Fish school density and volume. Mar. Biol. 54: 383–394.

RAMANI, N., P. H. PATRICK, W. H. HANSON, AND H. ANDERSON. 1989. Fish detection and classification using a neural network based active sonar system — preliminary results. Int. Joint Conference on Neural Networks — Proceedings.

ROSE, G. A., AND W. C. LEGGETT. 1988. Hydroacoustic signal classification of fish schools by species. Can. J. Fish. Aquat. Sci. 45: 597–604.

TESLER, W. D. 1989. Bias and precision in acoustic biomass estimation. Proc. Inst. Acoust. 3: 202–211.

THORNE, R. E. 1988. An empirical evaluation of the duration-in-beam technique for hydroacoustic evaluation. Can. J. Fish. Aquat. Sci. 45: 1244–1248.

TRAYNOR, J. J., AND J. E. EHRENBERG. 1979. Evaluation of the dual beam acoustic fish target strength measurement method. J. Fish. Res. Board Can. 36: 1065–1075.

Usefulness of Fourier Analysis of Otolith Shape for Atlantic Mackerel (*Scomber scombrus*) Stock Discrimination

Martin Castonguay

Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli (Québec) G5H 3Z4, Canada

Patrick Simard

Département de chimie-biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières (Québec) G9A 5H7, Canada

and Pierre Gagnon

Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli (Québec) G5H 3Z4, Canada

Castonguay, M., P. Simard, and P. Gagnon. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. Can. J. Fish. Aquat. Sci. 48: 296–302.

We compared shapes of Atlantic mackerel (*Scomber scombrus*) sagittae between the two contingents (i.e. spawning groups) from the Northwest Atlantic and between the stocks from the Northwest Atlantic and the North Sea to evaluate whether otolith shape could differentiate between the two contingents in a mixed fishery. We quantified shapes with the Fourier series, an objective and rapid method which decomposes a shape's outline into a series of sinusoids. To determine a correct way to compare contingents/stocks, we first assessed four intracontingent effects on otolith shapes. Age and year-class effects were significant, while sex and bilateral position effects were not. This temporal instability in shapes indicates that confounding effects of age and year-class on otolith shapes need to be assessed carefully before drawing conclusions on stock structure. It also shows that comparative studies of otolith shapes with Fourier descriptors are not useful for mackerel contingent discrimination. The power of discriminant functions to correctly classify test mackerel samples separated by age and sampling year, on the basis of otolith shape, was better for comparisons between the stocks than for those between the contingents.

Nous avons comparé la forme de sagittae de maquereau bleu (*Scomber scombrus*) entre les deux contingents (i.e. groupes reproducteurs) du nord-ouest de l'Atlantique et entre les stocks du nord-ouest de l'Atlantique et de la mer du Nord afin de déterminer s'il est possible de différencier les deux contingents sur la base des formes d'otolithes dans une pêche mixte. Nous avons quantifié les formes à l'aide des séries de Fourier, une méthode objective et rapide qui décompose le contour d'une forme en une série de sinusoïdes. Afin de déterminer une façon correcte de comparer les contingents/stocks, nous avons d'abord évalué quatre effets intra-contingents sur les formes d'otolithes. Les effets d'âge et de classe d'âge sur les formes d'otolithes étaient significatifs alors que les effets de sexe et de position bilatérale ne l'étaient pas. Cette instabilité temporelle des formes indique que les effets d'âge et de classe d'âge doivent être soigneusement évalués avant de tirer des conclusions sur la structure de stock et démontre que des comparaisons de formes d'otolithes avec les descripteurs de Fourier ne sont pas utiles pour la discrimination des contingents de maquereau. La puissance de fonctions discriminantes à classifier correctement des échantillons tests de maquereau séparés par âge et année d'échantillonnage sur la base des formes d'otolithes était meilleure pour les comparaisons entre les stocks que pour celles entre les contingents.

Received January 22, 1990
Accepted August 10, 1990
(JA445)

Reçu le 22 janvier 1990
Accepté le 10 août 1990

Atlantic mackerel (*Scomber scombrus*) from the Northwest Atlantic has historically been divided between a "northern contingent" spawning in the Gulf of St. Lawrence in June and July and a "southern contingent" spawning on the continental shelf between Cape Cod and Cape Hatteras in April and May (Sette 1943, 1950). The two contingents (i.e. spawning groups) are managed as a single transboundary stock because large catches are made in the wintering area on the continental shelf where the two spawning groups cooccur (Maguire et al. 1987). The introduction of a rapid method of differentiation between the two contingents that would allow estimation of the relative proportion of each contingent harvested by the winter fishery would permit the development of alternate management strategies. Although the mean otolith area to the first annulus is significantly different between the two contingents, distribution of otolith areas overlap greatly (Grégoire and Castonguay 1989). Using otolith morphometrics to discriminate between stocks of Atlantic mackerel from the Northeast Atlantic, Hopkins (1986) reported that linear measurements exhibited a high degree of temporal variability and suggested that otolith shapes may be more temporally stable.

Jarvis et al. (1978) proposed a new method of shape characterization of scales to differentiate between fish stocks based on Fourier series. Fourier series are mathematically defined as a series of sinusoids (referred to here as Fourier descriptors, also called harmonics elsewhere) and can be used to objectively and rapidly describe the outline of a shape. Each Fourier descriptor is characterized by an amplitude and a phase angle.

TABLE 1. Numbers of fish per sampling year and age group from the different contingents/stocks on which Fourier descriptors of sagittae were measured.

Contingent/ stock	Sampling year	Age 1	2	3	4	5	Total
Northern	1983	4	8	8	6	14	40
	1984	0	65	54	15	0	134
	1985	11	8	166	34	0	219
	1987	30	100	21	8	60	219
Southern	1983	0	3	4	2	6	15
	1984	0	32	42	10	0	84
	1985	0	14	117	22	25	178
	1987	42	64	14	4	31	155
North Sea	1984	0	1	46	28	6	81
	1987	20	36	35	2	0	93
Total		107	331	507	131	142	1241

Being orthogonal, amplitudes of Fourier descriptors are amenable to various types of multivariate analysis.

Jarvis et al. (1978) were able to assign to the correct stock 80% of 40 walleye (*Stizostedion vitreum vitreum*) from two presumed stocks. By contrast, Riley and Carline (1982) concluded that differences in Fourier descriptors of scale shapes were insufficient to assign individual walleye to their stock of origin. Descriptions of otolith shapes using Fourier series allowed some separation among lake whitefish (*Coregonus clupeaformis*) stocks by basin of origin (Casselman et al. 1981). Bird et al. (1986) found that Fourier descriptors of herring (*Clupea harengus*) otoliths reflected differences in fish age and race. Pontual and Prouzet (1987, 1988) obtained a high degree of discrimination among different Atlantic salmon (*Salmo salar*) stocks using elliptic Fourier descriptors and other shape features of scales.

The objective of this study was to evaluate the usefulness of a stock discrimination approach for mackerel based on otolith shape using Fourier descriptors of sagittae (FDS). We used a three-step approach. (1) To determine a correct way to compare FDS between the two mackerel contingents, we carried out separate analyses of the effects of age, year-class, sex, and bilateral position on FDS. (2) We then conducted comparisons of FDS between the two contingents in a manner dictated by the findings of Step 1 to determine whether FDS were significantly different between contingents. The comparisons in Step 2 did not aim to elucidate whether the two contingents do indeed represent two genetic stocks, but rather to develop a rapid method of differentiation between the two contingents based on otolith shape, regardless of whether shape differences are genetically or environmentally induced. (3) Finally, we performed control comparisons of FDS between Atlantic mackerel from the Northwest and the Northeast Atlantic (North Sea stock) to assess how much variability in FDS should be expected in the case of stocks that we know to be genetically discrete.

Materials and methods

Sampling

Sagittae of mackerel from 1 to 5 yr old were randomly selected from commercial fishery samples collected by the Department of Fisheries and Oceans of Canada and the National Marine Fisheries Service of the United States in the Northwest Atlantic in 1983, 1984, 1985, and 1987 (Table 1). The mackerel from the southern contingent were captured on the eastern continental shelf at the time of spawning of that contingent (April–May), thus ensuring that they were southern contingent fish. The mackerel from the northern contingent were caught in the Gulf of St. Lawrence between June and October. Southern contingent mackerel do not occur in the Gulf of St. Lawrence (Sette 1950).

Sagittae of mackerel 1–5 yr old from the North Sea stock came from the sampling that the Institute of Marine Fisheries of Norway conducted in the North Sea and Skagerrak in June and August of 1984 and 1987 (Table 1). Fish age was determined with otoliths by experts of each country involved.

Calculation of Fourier Descriptors of Sagittae

Any function $R(\Theta)$ can be described by a Fourier series (Ehrlich and Weinberg 1970):

$$(1)\quad R(\Theta) = \bar{r} + \sum_{j=1}^{n/2-1} \{a_j\cos(j\Theta) + b_j\sin(j\Theta)\}.$$

In our study, $R(\Theta)$ is the radial length from the centre to the perimeter of the sagitta at an angle of rotation Θ, $\bar{r}$ is the mean radius, a_j and b_j are the Fourier coefficients of the jth Fourier descriptor, and n is the number of radii measured around the shape. Equation (1) can be reduced to

$$(2)\quad R(\Theta) = A_0 + \sum_{j=1}^{n/2-1} A_j\cos(j\Theta - \phi_j)$$

where $A_0 = \bar{r}$, A_j, and ϕ_j are the amplitude and the phase angle of the jth Fourier descriptor, respectively, and the other parameters are the same as above (Christopher and Waters 1974). The Fourier coefficients of Equation (1) are calculated as such:

$$(3)\quad a_j = 2{\cdot}n^{-1} \sum_{i=1}^{n} \{r_i\cos(j\Theta_i)\} \quad j = 1 \text{ to } n/2-1$$

$$(4)\quad b_j = 2{\cdot}n^{-1} \sum_{i=1}^{n} \{r_i\sin(j\Theta_i)\} \quad j = 1 \text{ to } n/2-1$$

where r_i is the length of the ith radius and Θ_i is the rotation angle corresponding to the ith radius (Christopher and Waters 1974). Amplitudes of Fourier descriptors (the A_j's of Equation (2)) can be described in terms of Fourier coefficients in the following way (Christopher and Waters 1974):

$$(5)\quad A_j = (a_j^2 + b_j^2)^{0.5}.$$

Low-order Fourier descriptors describe gross shape features, while high-order ones describe the finer details of the outline.

The A_j's (amplitudes) were the FDS used in this study. They were calculated with the Optical Pattern Recognition System (OPRS) software package from Biosonics. The OPRS, installed on a microcomputer, utilized a frame grabber (grid of 512 × 512 pixels) that digitized a video image of a given sagitta obtained from a camera coupled to a stereomicroscope at 12× magnification. The digitized image was then displayed and contrast-enhanced on a high-resolution monitor to obtain the best possible contrast between the sagitta and the background. The sagittal outline was digitized by the OPRS from the image. The 32 Fourier coefficients (sines and cosines), corresponding to 64 radii taken at regular angles from the shape centroid, were then generated. We computed amplitudes from the Fourier

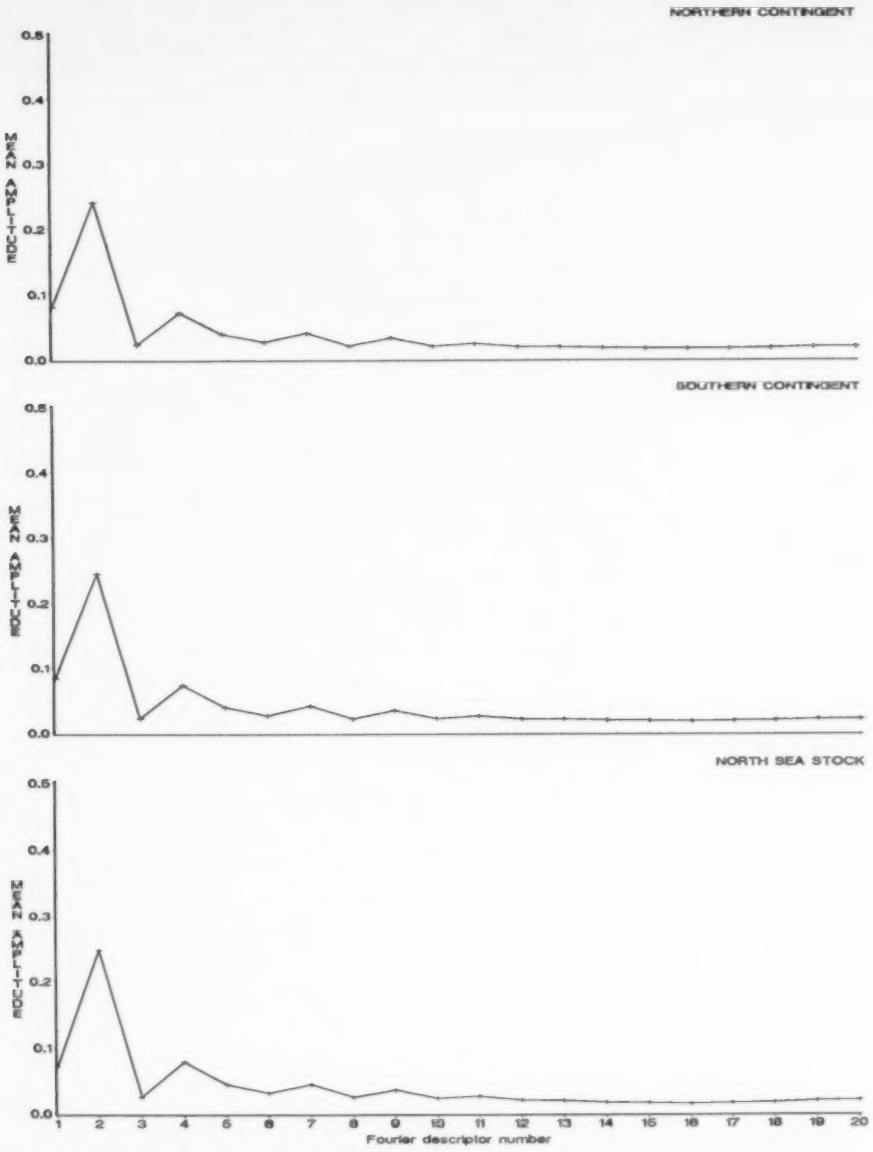


FIG. 1. Mean size-standardized Fourier descriptors of sagittae for the northern and southern contingents of Northwest Atlantic mackerel and for the North Sea stock.

coefficients, according to Equation (5). Only the first 20 amplitudes were used as shape descriptors (not counting the zeroth amplitude). Amplitudes were standardized for size by dividing each amplitude by the zeroth amplitude.

The centroid, calculated from an OPRS algorithm, was used as the otolith centre. The nucleus could not be used as the otolith centre because otoliths were often too opaque to pinpoint the nucleus. Interpretation of phase information depends upon the location of the centre. Because the centroid was unrelated to otolith features, resultant phase angle spectra were independent of outline morphology (Gevirtz 1976) and were therefore not used.

Experimental Design and Statistics

The amplitude spectrum of the first 20 Fourier descriptors of individual sagittae were the FDS data used for analysis. To conduct meaningful comparisons between contingents/stocks, we first assessed four intracontingent effects on FDS using univariate (ANOVA) and multivariate (MANOVA) analyses of variance. Those intracontingent effects were (1) age effect (comparison of different ages from the same sampling year), (2) year-class effect (comparison of same age sampled in different years), (3) sex effect, and (4) bilateral position effect.

FDS comparisons between contingents/stocks were conducted with ANOVA's, MANOVA's, and discriminant analyses to determine whether FDS differed significantly. Discriminant analyses were only calculated in comparisons for which the sample size exceeded 99. In such cases, the otoliths were divided into two approximately equal subsamples on the

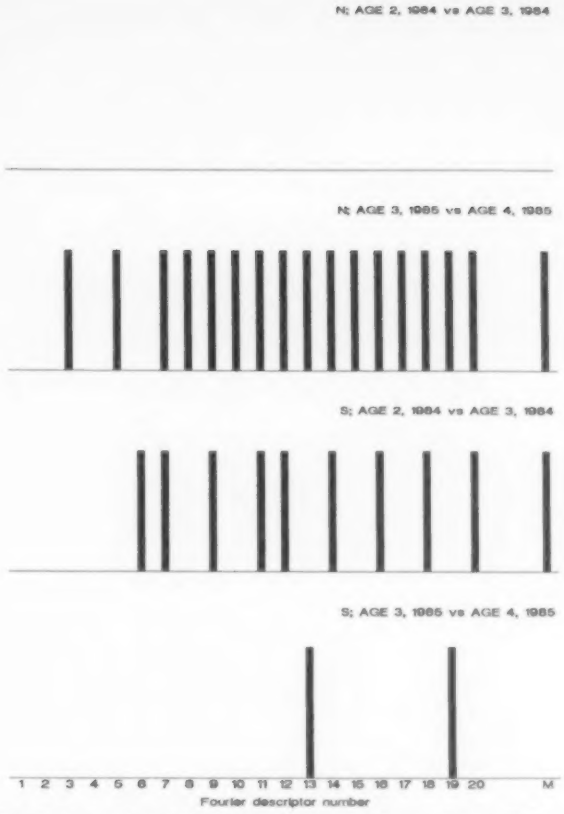


FIG. 2. Uni- and multivariate analyses of variance of age effect on Fourier descriptors of sagittae (FDS) for standardized comparisons within the northern contingent (N) and within the southern contingent (S). Presence of a bar indicates significance of individual FDS (1–20) (ANOVA's, $P < 0.05$) and overall significance (M) (MANOVA, $P < 0.05$).

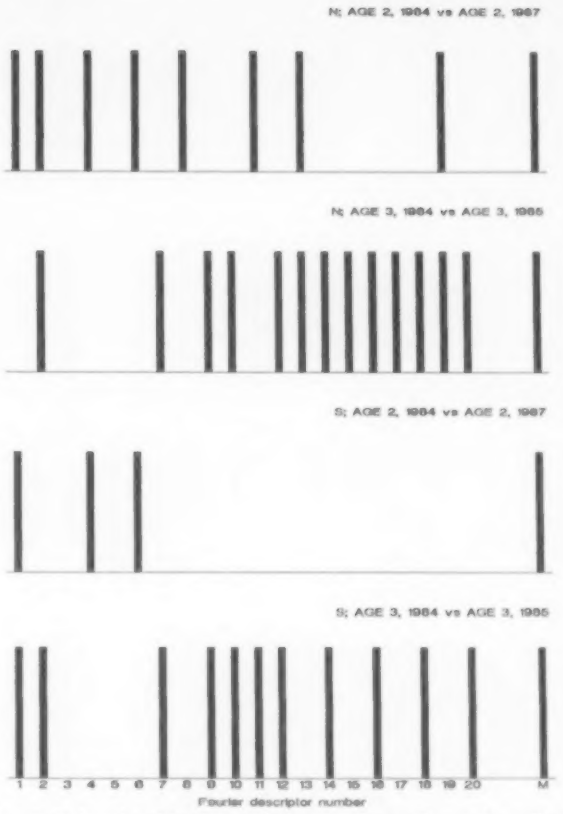


FIG. 3. Uni- and multivariate analyses of variance of year-class effect on Fourier descriptors of sagittae (FDS) for standardized comparisons within the northern contingent (N) and within the southern contingent (S). Presence of a bar indicates significance of individual FDS (1–20) (ANOVA's, $P < 0.05$) and overall significance (M) (MANOVA, $P < 0.05$).

basis of a binary variable (odd or even position number on the holding tray). One subsample was used to build the discriminant function, while the power of the function was evaluated with the other subsample (referred to here as test sample). A discriminant function builds a set of linear combinations that best reveals differences among groups. Classification of the test sample provides a measure of the general utility of the discrimination when extended to the whole population.

The relative contribution of each FDS to the discrimination, expressed as partial coefficients of determination (r^2), was examined with forward stepwise discriminant analyses incorporating a subset from the 20 FDS that yielded the best discrimination possible. Most individual FDS had normal distributions (Shapiro–Wilk tests, $P > 0.05$) and variances that were homogeneous between contingents/stocks (F_{max} tests, $P > 0.05$ (Sokal and Rohlf 1981)). Statistical analyses were carried out with the package SAS (SAS Institute Inc. 1987). For mackerel sampled in 1983, the two sagittae of each fish were analysed. For mackerel captured in subsequent years, the left sagitta was used if undamaged; otherwise the right sagitta was used.

Results

Intracontingent Effects

To determine a correct way to statistically compare the FDS value spectra presented in Fig. 1 between contingents/stocks, age, year-class, sex, and bilateral position effects on FDS were assessed. Age had a significant effect on sagittae shapes in two of four comparisons (one comparison per contingent) between age groups sampled the same year. For these two comparisons, significant FDS differences occurred between age groups when the 20 FDS were considered simultaneously (MANOVA's, $P < 0.02$; Fig. 2), and 16 and 9 individual FDS were significantly different between age groups (ANOVA's, $P < 0.05$; Fig. 2).

The year-class effect was significant in the four comparisons of the same age between sampling years, as we found significant differences of FDS values when the 20 FDS were considered simultaneously (MANOVA's, $P < 0.003$; Fig. 3). Eight and 13 FDS of the two comparisons within the northern contingent, and 3 and 11 FDS of the two comparisons within the southern contingent were significantly different between years (ANOVA's, $P < 0.05$: Fig. 3).

No FDS differences were observed in the two comparisons between male and female mackerel of a given year-class and

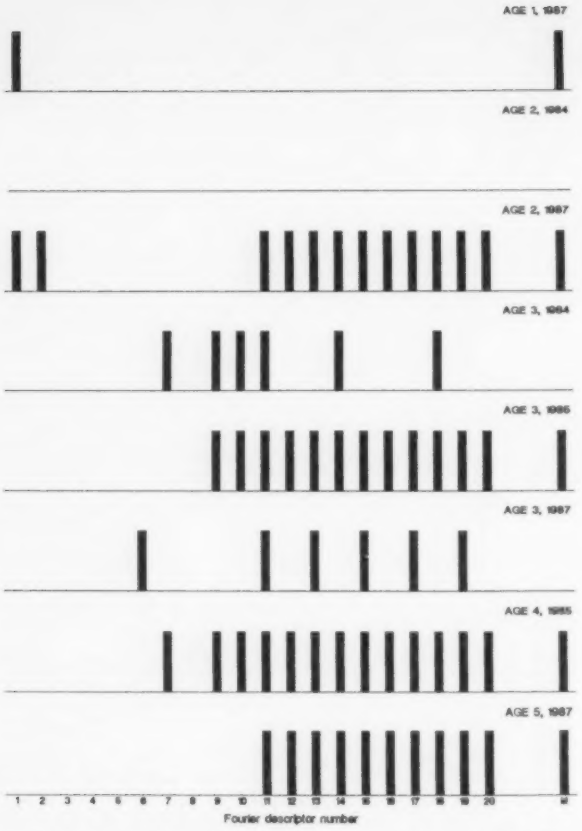


FIG. 4. Uni- and multivariate analyses of variance of contingent effect on Fourier descriptors of sagittae (FDS) for standardized comparisons between the northern contingent and the southern contingent. Presence of a bar indicates significance of individual FDS (1–20) (ANOVA's, $P < 0.05$) and overall significance (M) (MANOVA, $P < 0.05$).

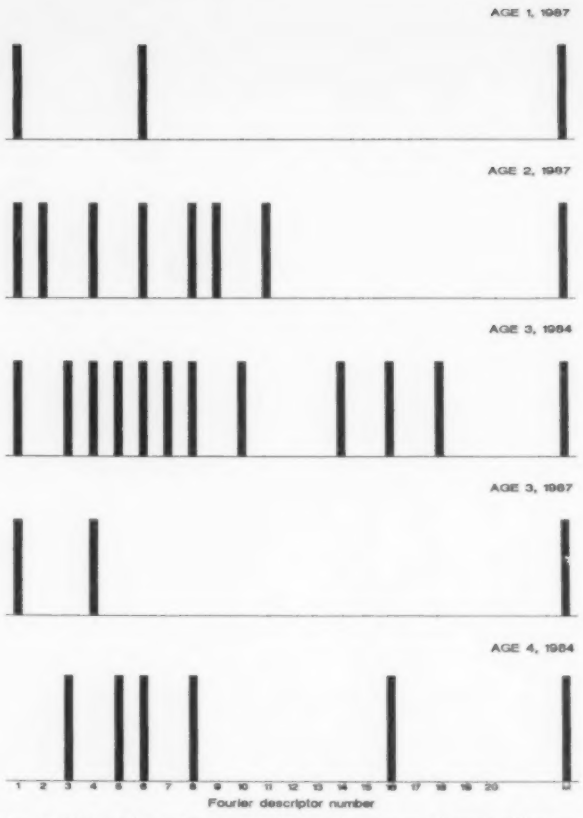


FIG. 5. Uni- and multivariate analyses of variance of stock effect on Fourier descriptors of sagittae (FDS) for standardized comparisons between the northern contingent and the North Sea Stock. Presence of a bar indicates significance of individual FDS (1–20) (ANOVA's, $P < 0.05$) and overall significance (M) (MANOVA, $P < 0.05$).

sampling year when the 20 FDS were considered simultaneously (MANOVA's, $P > 0.05$). No difference was found either between the left and the right sagitta when the 20 FDS were considered simultaneously (MANOVA, $P > 0.05$).

Intercontingent/Stock Effects

The previous analyses, especially the MANOVA's, indicated that comparisons between contingents/stocks have to be standardized for age and year-class effects. The contingent/stock comparisons were therefore separated by age and sampling year.

Of the eight comparisons between the northern and the southern contingent, five had significant overall FDS value differences (MANOVA's, $P < 0.02$; Fig. 4). For these five comparisons the number of significantly different FDS was highly variable among comparisons, as it ranged from 1 to 13 (ANOVA's, $P < 0.05$; Fig. 4).

In the five comparisons between the Northwest Atlantic stock and the North Sea stock, we found significant overall differences of FDS values (MANOVA's, $P < 0.03$; Fig. 5). Between 2 and 11 FDS were different between the two stocks (ANOVA's, $P < 0.05$; Fig. 5).

The percentage of correct classification of the test sample by the discriminant functions calculated on two intercontingent comparisons was only in the 36–68% range (Table 2A), even though the overall difference between contingents was highly significant for these two comparisons (MANOVA's, $P < 0.002$; Fig. 4). For interstock comparisons, however, that percentage was higher, in the 60–91% range (Tables 2B and 2C). The examination of relative contributions of individual

TABLE 2. Percentage of correct classification of the test sample by the discriminant functions calculated on Fourier descriptors of sagittae.

Comparison	% of correct classification	
(A) Northern vs. southern contingent	Northern	Southern
1985, age 3	67.8	35.6
1987, age 2	64.7	46.9
(B) Northern contingent vs. North Sea stock	Northern	North Sea
1984, age 3	89.7	85.0
1987, age 2	82.4	60.0
(C) Southern contingent vs. North Sea stock	Southern	North Sea
1987, age 2	90.6	86.7

TABLE 3. Relative contribution (expressed by the partial coefficients of determination (r^2)) of the Fourier descriptors of sagittae (FDS) that contributed the most to the discrimination between contingents ($P < 0.05$).

Comparison	FDS number	Partial r^2
1984, age 3	1	0.057
1985, age 3	1	0.027
	10	0.037
	13	0.009
1985, age 4	16	0.190
	15	0.052
	14	0.045
1987, age 2	2	0.076
	4	0.043
	3	0.032
1987, age 5	14	0.105
	1	0.046
	8	0.087

TABLE 4. Relative contribution (expressed by the partial coefficients of determination (r^2)) of the Fourier descriptors of sagittae (FDS) that contributed the most to the discrimination between stocks ($P < 0.05$).

Comparison	FDS number	Partial r^2
Northern contingent vs. North Sea stock		
1984, age 3	1	0.124
	8	0.403
	11	0.076
1984, age 4	6	0.211
	16	0.108
	18	0.098
1987, age 2	1	0.066
	8	0.059
	15	0.026
Southern contingent vs. North Sea stock		
1984, age 3	1	0.215
	8	0.307
	5	0.165
1987, age 2	1	0.149
	8	0.233
	4	0.138

FDS (expressed by the partial r^2) to discriminate between contingents (Table 3) or between stocks (Table 4) showed that differences were found in low-order as well as in high-order FDS.

Discussion

This study emphasizes the need to carefully evaluate confounding age and especially year-class effects on Fourier descriptors of otoliths before drawing conclusions about stock structure on the basis of shape differences among samples of fish from different stocks. Not accounting for age and year-class effects may result in falsely attributing shape differences between samples to a stock effect, while they may in fact reflect differences in age structure and/or of sampling year between the samples. In other words, differences in Fourier descriptors of otoliths or scales among samples from different stocks may tend to highlight sample differences rather than stock differences. Bird et al. (1986) also found age effects on FDS of herring and reported few differences between right and left otoliths and between sexes. Our results contrast with those of Riley and Carline (1982) and Pontual (1986) who did not find significant intrastock effects on Fourier descriptors of walleye and Atlantic salmon scales, respectively.

This study shows that mean sagittal shape may change with age and also between year-classes. The temporal instability in otolith shape among mackerel of a given contingent, as expressed by Fourier analysis, shows that comparative studies of FDS are not useful for mackerel contingent discrimination. Even if discriminations calculated on intercontingent comparisons separated by age and sampling year had yielded a high level of correct classification, one would still have to adopt the impractical approach of recalculating discriminant functions every year for each of the major age groups present in the fishery. The unit for intercontingent comparisons would have to be a given age group from a given sampling year, as age and year-class effects on FDS (as expressed by the MANOVA's) were significant in most comparisons. These significant overall effects were the result of individual differences (ANOVA's) in low-order FDS as well as in high-order FDS, indicating that differences in gross shape and in finer details of the outline of otoliths were present. The results of the stepwise discriminant analyses argue similarly.

Otolith shapes appeared to be more distinct in comparisons between stocks than in comparisons between contingents, as the performance of the discriminant analysis to correctly classify mackerel on the basis of FDS was better in the former. This may indicate that the two contingents are less genetically distinct from each other than are the stocks from either side of the Atlantic Ocean and/or that environmental conditions are less different between the contingents than between the stocks. Although FDS differences between contingents (separated by age and sampling year) were not large enough to yield good discrimination, they were nevertheless statistically significant, as shown by the MANOVA's.

Shape characterization approaches are probably more appropriate in taxonomic studies than for stock identification. Jeffries et al. (1984) developed a system to automate the analysis of zooplankton based on discriminant analyses of morphological features. They obtained a 90% accuracy of classification of eight taxonomic groups. Fourier analysis of somatic shape allowed differentiation among taxa of fossil organisms such as ostracods (Kaesler and Waters 1972; Younker and Ehrlich 1977), miospores (Christopher and Waters 1974), and foraminifera (Healy-Williams and Williams 1981).

Acknowledgments

We thank Drs. W. J. Overholtz (U.S. National Marine Fisheries Service) and S. A. Iversen (Institute of Marine Research of Norway) for supplying otoliths. Drs. D. D'Amours, D. Gascon, and especially D. W. Welch provided useful comments on earlier drafts of the manuscript.

References

BIRD, J. L., D. T. EPPLER, AND D. M. CHECKLEY. 1986. Comparisons of herring otoliths using Fourier series shape analysis. Can. J. Fish. Aquat. Sci. 43: 1228–1234.

CASSELMAN, J. L., J. J. COLLINS, E. J. CROSSMAN, P. E. IHSSEN, AND G. R. SPANGLER. 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci. 38: 1772–1789.

CHRISTOPHER, R. A., AND J. A. WATERS. 1974. Fourier series as a quantitative descriptor of miospore shape. J. Paleontol. 48: 697–709.

EHRLICH, R., AND B. WEINBERG. 1970. An exact method for characterization of grain shape. J. Sediment. Petrol. 40: 205–212.

GEVIRTZ, J. L. 1976. Fourier analysis of bivalve outlines: implications on evolution and autecology. J. Int. Assoc. Math. Geol. 8: 151–163.

GRÉGOIRE, F., and M. CASTONGUAY. 1989. Etude de dimensions au premier annulus d'otolithes de maquereau bleu (*Scomber scombrus*) du nord-ouest de l'Atlantique. Can. Tech. Rep. Fish. Aquat. Sci. 1680: vi + 15 p.

HEALY-WILLIAMS, N., AND D. F. WILLIAMS. 1981. Fourier analysis of test shape of planktonic foraminifera. Nature (Lond.) 289: 485–487.

HOPKINS, P. J. 1986. Mackerel stock discrimination using otolith morphometrics. ICES CM 1986/H:7. 17 p.

JARVIS, R. S., H. F. KLODOWSKI, AND S. P. SHELDON. 1978. New method of quantifying scale shape and an application to stock identification in walleye (*Stizostedion vitreum vitreum*). Trans. Am. Fish. Soc. 107: 528–534.

JEFFRIES, H. P., M. S. BERMAN, A. D. POULARIKAS, C. KATSINIS, I. MELAS, K. SHERMAN, AND L. BIVINS. 1984. Automated sizing, counting and identification of zooplankton by pattern recognition. Mar. Biol. 78: 329–334.

KAESLER, R. L., AND J. A. WATERS. 1972. Fourier analysis of the ostracode margin. Geol. Soc. Am. Bull. 83: 1169–1178.

MAGUIRE, J.-J., Y. C. CHAGNON, M. CASTONGUAY, AND B. MERCILLE. 1987. A review of mackerel management areas in the Northwest Atlantic. CAFSAC Res. Doc. 87/71: 31 p.

PONTUAL, H. DE. 1986. Essais de discrimination de stocks de saumon Atlantique (*Salmo salar*) par reconnaissance de la forme de leurs écailles. Ph.D. thesis, Université Pierre et Marie Curie, Paris. 167 p.

PONTUAL, H. DE, AND P. PROUZET. 1987. Atlantic salmon, *Salmo salar* L., stock discrimination by scale-shape analysis. Aquacult. Fish. Manage. 18: 277–289.

1988. Numerical analysis of scale morphology to discriminate between Atlantic salmon stocks. Aquat. Living Res. 1: 17–27.

RILEY, L. M., AND R. F. CARLINE. 1982. Use of scale shape for identification of walleye stocks from western Lake Erie. Trans. Am. Fish. Soc. 111: 736–741.

SAS INSTITUTE INC. 1987. SAS user's guide: statistics. Version 5. SAS Institute Inc., Cary, NC. 956 p.

SETTE, O. E. 1943. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part I: Early life history, including the growth, drift, and mortality of the egg and larval populations. U.S. Fish Wildl. Serv. Fish. Bull. 50: 149–237.

1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part II: Migrations and habits. U.S. Fish Wildl. Serv. Fish. Bull. 51: 251–358.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman, San Francisco, CA.

YOUNKER, J. L., AND R. EHRLICH. 1977. Fourier biometrics: harmonic amplitudes as multivariate shape descriptors. Syst. Zool. 26: 336–342.

Comparison of Catch Rates among Small and Large Bongo Samplers for *Calanus finmarchicus* Copepodite Stages

John T. Anderson and William G. Warren

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Nfld. A1C 5X1, Canada

Anderson, J. T., and W. G. Warren. 1991. Comparison of catch rates among small and large bongo samplers for *Calanus finmarchicus* copepodite stages. Can. J. Fish. Aquat. Sci. 48: 303–308.

Comparison of catch rates between small (20-cm, 0.165-mm-mesh nets) and large (61-cm, 0.333- and 0.505-mm-mesh nets) plankton samplers was carried out for *Calanus finmarchicus* copepodite stages sampled simultaneously during six different times. It is concluded that *C. finmarchicus* stages CI and CII were undersampled in 0.333-mm-mesh samples, while stages CIV–CVI were undersampled in 0.165-mm-mesh samples. Stage CIII catch rates were not different for these two samplers. Comparison of catch rates between 0.165- and 0.505-mm-mesh samples demonstrated that stages CI–CIII were undersampled in the 0.505-mm-mesh samples, stages CV and CVI were undersampled in the 0.165-mm-mesh samples, and there was no difference in catch rates for stage CIV. There was evidence of substantial variability in results among cruises for most stages. This is attributed to inherent variability associated with plankton sampling due to their patchy distribution. Due to the large variation in catch rates between these different samplers, it is recommended that the appropriate sized sampler be used to collect different stages of *C. finmarchicus* copepodites rather than attempting to correct for catch rate differences among different sized samplers.

On a comparé les taux de capture obtenus à l'aide de petits (filets de 20 cm à maillage de 0,165 mm) et de gros (filets de 61 cm à maillage de 0,333 et 0,505 mm) appareils d'échantillonnage des stades copépodites de *Calanus finmarchicus* capturés simultanément à six différentes occasions. On a formulé la conclusion que le maillage de 0,333 mm ne donne pas une échantillon représentatif des stades CI et CII de *C. finmarchicus* et qu'il en était de même pour le maillage de 0,165 mm dans le cas des stages CIV et CVI. Par contre, les taux de capture du stade CIII ne variaient pas en fonction de ces maillages Une comparaison des taux de capture obtenus à l'aide des maillages de 0,165 mm et 0,505 mm a révélé que les échantillons obtenus à l'aide du maillage de 0,505 mm n'étaient pas représentatifs des stades CI–CIII, que le maillage de 0,165 mm n'a pas donné des échantillons représentatifs des stades CV et CVI et que les taux de capture du stade CIV étaient semblables. On a noté une importante variabilité nette des résultats obtenus au cours des croisières d'inventaire dans le cas de la plupart des stades. Ceci est imputé à la variabilité propre à l'échantillonnage du plancton attribuable à l'inégalité de sa répartition. À cause de la grande variation des taux de capture de ces différents maillages, il est recommandé que le maillage approprié soit utilisé pour la collecte des différents stades copépodites de *C. finmarchicus* et non de tenir compte des différences entre les taux de capture obtenus à l'aide des différents maillages.

Received January 24, 1990
Accepted August 10, 1990
(JA447)

Reçu le 24 janvier 1990
Accepté le 10 août 1990

Estimating production in the ocean is a major focus of marine research. Measurements of secondary production of copepods in the ocean is difficult, but necessary to provide flux estimates crucial to understanding ecosystem function (Kimmerer 1987). Several methods exist for estimating secondary production. Methods for directly estimating secondary production using either the cohort method of short-term experiments (e.g. McLaren et al. 1989) require accurate sampling of different development stages of copepods.

Calanus finmarchicus numerically dominates the invertebrate zooplankton of oceanic waters throughout the North Atlantic, paticularly during spring when the species usually dominates secondary production. Specific studies demonstrating this dominance in the Northwest Atlantic include those in Davis Strait (Bainbridge and Corlett 1968; Huntley et al. 1983), waters off both East and West Greenland, the Irminger Sea and the Labrador Sea (Bainbridge and Corlett 1968), Flemish Cap (Anderson 1990), the Scotian Shelf (Tremblay and Roff 1983), and on Georges Bank (Davis 1987). Similarly, *C. finmarchicus* dominates waters in the North Sea and sometimes accounts for up to 70% of the zooplankton biomass (Williams and Lindley 1980; Krauss and Trahms 1983). Plankton recorder surveys carried out over many years on transects throughout the North Atlantic demonstrate that *C. finmarchicus* dominance is a feature that persists over many years (Glover 1967; Glover and Robinson 1968).

Development of *C. finmarchicus* ranges from eggs 0.146–0.151 mm in diameter (McLaren et al. 1988) to copepodites 1.74–2.03 mm total length at stage CV (Conway and Minton 1975) when diapause typically occurs. This represents a size range of approximately 1.5 orders of magnitude. The copepodite stages alone change by a factor of 3.5 from CI to CVI (female) (Conway and Minton 1975). Due to the rapid development of copepod species through all development stages (at least to diapuse), it is usually necessary to sample *C. finmarchicus* with more than one mesh size to ensure that it is accurately sampled. In many studies, however, copepod stages are sampled by only one mesh size and estimates of production are derived from these data (Davis 1987; McLaren et al. 1989). Only in the case of Tremblay and Roff (1983) were two mesh

TABLE 1. Summary of cruises and samples processed for analyses of catch rate differences in *C. finmarchicus* copepodite stages on Flemish Cap.

Cruise No.	Date	61-cm bongo		20-cm bongo,
		0.505-mm mesh	0.333-mm mesh	0.165-mm mesh
GAD035	7–14 April 1980	—	40	39
GAD037	21–31 May 1980	—	56	41
GAD050	2–9 May 1981	31	10	41
GAD051	22–27 May 1981	—	42	41
HAWPAN	26–30 June 1981	—	42	44

sizes used and a comparison of retention made for copepod species of different sizes. The purpose of this paper is to present results of an analysis of catch rate differences for *C. finmarchicus* copepodites sampled by small (20-cm, 0.165-mm-mesh nets) and large (61-cm, 0.333- and 0.505-mm-mesh nets) bongo samplers typically used in marine research (Smith and Richardson 1977).

Methods

Catch rate data for 0.165- and 0.333-mm-mesh nets were compared for six cruises on Flemish Cap (47°N, 45°W) in which the two mesh sizes were sampled simultaneously on 20- and 61-cm bongo samplers, respectively (Table 1). In one cruise, 31 tows were also done using 0.505-mm-mesh nets in place of the 0.333-mm-mesh nets. The bongo sampler was towed obliquely from near the bottom or 200 m depth, whichever was less, at ~1.25–1.5 $m \cdot s^{-1}$. Payout and retrieval rates were 0.83 and 0.33 $m \cdot s^{-1}$, respectively. The complete sampling outline is described in Anderson (1990). The mean catch rate (numbers per cubic metre) was calculated for each copepodite stage (CI–CVI) in each mesh size for each of the six cruises.

The numbers of zooplankton caught by each tow within a cruise often exhibited strong positive skewness and the assumptions needed to validate the conventional paired *t*-test for the comparison of the catches between samplers, for each copepodite stage, appeared to be violated to the extent that the results could be misleading. Therefore, we decided to use the Wilcoxon matched-pairs signed-ranks statistic to test the null hypothesis that there was no difference in catchability between the mesh sizes (e.g. see Siegel 1956). This test is not dependent on any distributional assumptions.

Since, a priori, it was suspected that the 0.165-mm-mesh samples would retain more of the smaller copepodite stages than the 0.333-mm (or 0.505-mm) mesh samples, but that the reverse would apply to the larger stages, the tests were one-sided against the appropriate alternative. The difficulty that arises with the intermediate copepodite stages was resolved by testing against the reverse alternative if the null hypothesis was accepted over the initially selected alternative.

For the comparison of the 0.165- and 0.333-mm-mesh samples, for each copepodite stage, one has an independent test of the same null hypothesis from each of the six cruises. The outcomes of these six tests should not be looked at in isolation from each other; fortunately, there exist means by which they may be formally combined for the purpose of generating an overall interpretation. In recent years the term "meta-analysis" has been coined to cover this aspect of statistical inference (e.g. see Hedges and Olkin 1985).

The oldest of these approaches, generally ascribed to Fisher (1932) and thus preceding the term "meta-analysis," in effect utilizes the geometric mean of the resulting *p*-values. Under the assumption of no difference in catch rates between two nets, the individual *p*-values (p_i) should be uniformally distributed on (0,1) whence $-2\sum_{i=1}^{n} \log(p_i)$ would be distributed as a χ^2 with $2n$ degrees of freedom where here, n is the number of cruises (6). Accordingly this statistic has been calculated for each copepodite stage.

One may also use any recognized goodness-of-fit test to assess whether the individual *p*-values could have reasonably arisen from a uniform (0,1) distribution; of such tests, the one used here, namely that due to Anderson and Darling (1952), A^2, is convenient and has been found elsewhere to be amongst the more powerful against a wide range of alternatives.

Rejection of the null hypothesis by the Anderson–Darling test can be due to (i) a preponderance of *p*-values either above or below their expected value of 0.5 (indicating a departure from the hypothesis of no difference in one direction or the other) or (ii) a surplus of both high and low values of p, indicating inconsistency and reflected by the variance of the *p*-values being greater than expectation (namely 1/2) or (iii) a combination of these situations.

The above χ^2 test is but one way of testing whether explanation (i) above is the appropriate alternative. The quantity $P = (\sum_{i=1}^{n} p_i)^n/n!$ provides an exact test of the hypothesis $E(p) = 0.5$ provided $\sum_{i=1}^{n} p_i < 1$ (Edgington 1972). It becomes progressively less viable, and may even appear ridiculous, when $\sum_{i=1}^{n} p_i > 1$. However, for sufficiently large n (>5, say), under the null hypothesis, $z = \sqrt{12n}(\bar{p} - 0.5)$, with $p = \sum_{i=1}^{n} p_i/n$, is for all practical purposes distributed as a standard normal variate.

The notion of inconsistency, although important ((ii) above), has not been well addressed in the literature and as yet there is no viable, formal test procedure.

Note that when the Wilcoxon test statistic is calculated from more than 25 pairs it may be transformed to a quantity that, under the null hypothesis, closely follows a standard normal distribution, thus permitting the ready determination of actual *p*-values. In the study, zero catches of a particular stage in both meshes has been interpreted as an absence of that stage of *C. finmarchicus*, rather than as evidence of equal catchability, and therefore, such instances have been omitted from the analysis. In some cases this has resulted in less than 25 pairs remaining for the cruise–stage combination. Comprehensive tables of *p*-values, in contrast with critical values at conventional probability levels, are not readily available. When the number of pairs is small (<10, say) or the value of the Wilcoxon statistic is small (<5, say), exact *p*-values can be readily computed by means of a recursion formula. Unfortunately, there remain a few cases where this approach is unduly laborious, e.g. GAD035 CII, with 17 pairs and Wilcoxon's $T = 58$. For these cases the normal approximation was again used. Monte Carlo simulations indicated that the error in so doing would have a negligible effect on the analyses that followed.

Results

Analysis of catch rate differences between small and large bongo samplers clearly demonstrated that *C. finmarchicus* copepodite stages CI and CII were undersampled by the large bongos fitted with 0.333-mm-mesh nets, while stages CV and CVI were undersampled by the small bongos. These results are

TABLE 2. Comparison of catch rate differences between 0.165- and 0.33-mm-mesh nets for different copepodite stages of *C. finmarchicus* sampled during six cruises.

		0.165-mm mesh		0.333-mm mesh			
	N	Mean	Skewness	Mean	Skewness	Wilcoxon *T*	Probability (one-sided)
Stage CI							
ATC331	10	3.6143	1.075	0	—	0 (27)	1/1024 = 0.00098
GAD035	12	0.9168	2.466	0.0162	3.464	0 (33)	6/4096 = 0.00146
GAD037	35	19.3426	5.091	0.8146	1.969	29 (315)	0.0000014
GAD050	8	93.7625	2.797	0	—	0 (18)	1/256 = 0.0039
GAD051	15	10.9947	2.030	0	—	0 (60)	1/32768 = 0.00003
HAWPAN	7	16.1184	1.362	0	—	0 (14)	1/128 = 0.0078
Stage CII							
ATC331	8	2.5910	2.558	0	—	0 (18)	1/256 = 0.0039
GAD035	17	0.3914	2.929	0.1694	0.818	58 (76)	0.1906
GAD037	41	28.2278	2.419	12.1437	2.946	245 (430)	0.0081
GAD050	9	106.8197	1.508	0	—	0 (22)	1/512 = 0.00195
GAD051	28	8.0821	2.593	4.1343	2.148	94 (203)	0.0065
HAWPAN	6	8.2673	1.983	0	—	0 (10)	1/64 = 0.0156
Stage CIII							
ATC331	20	5.4699	3.123	6.4256	2.337	75 (105)[a]	0.1314
GAD035	29	0.2500	4.293	0.2357	3.312	171 (217)[a]	0.1573
GAD037	41	42.6613	1.227	49.8164	4.168	422 (430)[a]	0.4561
GAD050	9	142.0682	0.347	75.5482	1.489	9 (22)	33/512 = 0.06445
GAD051	41	44.2226	2.384	46.6323	2.315	430 (430)	0.4974
HAWPAN	30	3.1658	1.686	2.6500	2.108	211 (232)[a]	0.3292
Stage CIV							
ATC331	21	73.7041	1.472	85.5424	0.983	93 (115)	0.2171
GAD035	35	1.1327	2.222	1.3856	1.715	189 (315)	0.0196
GAD037	41	26.0747	1.950	31.1729	2.492	395 (430)	0.3228
GAD050	9	47.3430	1.852	104.8872	0.726	8 (22)	25/512 = 0.0488
GAD051	41	167.3330	1.495	199.4791	1.210	254 (430)	0.0111
HAWPAN	39	14.7989	2.003	15.3721	2.329	351 (390)	0.2931
Stage CV							
ATC331	21	326.4441	3.074	258.4392	1.739	86 (115)[b]	0.1526
GAD035	36	1.0756	2.373	1.0616	1.616	250 (333)	0.0961
GAD037	41	3.6157	2.757	4.4884	1.633	292 (430)	0.0363
GAD050	9	3.9643	1.672	9.5499	0.808	7 (22)	19/512 = 0.0371
GAD051	41	83.4466	1.397	103.6961	1.514	203 (430)	0.0016
HAWPAN	40	89.5095	1.570	98.1962	1.674	347 (410)	0.1986
Stage CVI							
ATC331	22	5.1645	0.790	6.9870	1.354	76 (126)	0.0849
GAD035	37	40.6106	1.565	46.2756	1.536	211 (351)	0.0170
GAD037	41	18.2187	0.978	24.9437	1.064	283 (430)	0.0300
GAD050	9	11.7379	2.378	18.1867	0.813	11 (22)	52/512 = 0.1016
GAD051	41	20.5782	2.302	23.1940	0.657	212 (430)	0.0023
HAWPAN	40	11.3607	1.944	11.8662	1.281	351 (410)	0.2139

[a]Directional change (i.e. catch 333 > catch 165).
[b]In reverse direction (i.e. catch 165 > catch 333).

apparent from the individual tests of differences within each cruise (Table 2) and from the overall results of the meta-analyses of all cruises for each of these stages (Table 3). The samples were processed by a different laboratory in 1980 than in 1981 and 1982. It appears that staging of CI and CII copepodites for the large bongos was not carried out for the 1981 and 1982 samples. This is suggested by zero catches in four cruises for stage CI and three cruises for stage CII which is considered unlikely compared with catches in the small sampler (Table 2). Therefore, we conclude that catch rate differences for stage CI and CII for these cruises are not valid. Nevertheless, the differences observed for two cruises for stage CI and three cruises for stage CII are convincing (Table 2).

Catch rate differences for stage CIII demonstrated no significant difference between samplers. This was true for all six cruises where probabilities associated with rejecting the null hypothesis ranged from 0.1314 to 0.4974. There was some variability in results for different cruises. For example, GAD050 data indicated that the small bongos caught almost twice as many stage CIII copepodites as the large bongos, compared with relatively small differences for all other cruises. The mean catch rate differences in GAD050 were not a result of an

TABLE 3. Summary of meta-analysis to test for catch rate differences for all cruises combined for each copepodite stage of *C. finmarchicus*. See Methods for explanation of each test.

Stage	χ^2 (p)	A^2	$\bar{p}$ z	P
CI	*99.04* (*8.6×10^{-16}*)	33.68	0.0022 4.2243	6.6×10^{-15}
CII	58.89 (1.9×10^{-7})	16.97	0.0378 3.9220	1.9×10^{-7}
CIII	13.06 (0.3647)	0.5534	0.4187 0.6899	0.3491
CIV	30.69 (0.0022)	5.4820	0.1521 2.9532	0.0008
CV	34.34 (0.0006)	5.5478	0.2029 2.5214	0.0045
CVI	39.90 (0.0001)	9.3398	0.0750 3.6067	0.000011

extreme value. Indeed, measures of skewness for these samples were lower compared with that sampled for other cruises (Table 2). The small sample size for this cruise may account for the observed difference which would tend to cancel out as more samples were included in the calculation of mean density.

Results for stage CIV are less clear. Overall we conclude there is a measureable difference in catch rates: the larger bongos caught more stage CIV copepodites than the small bongos. We base this conclusion on the results of the meta-analyses which demonstrated overall that we would reject the null hypothesis and on the observation that in all cases the mean catches of the large bongos were greater. For each cruise the differences between the means were not large, suggesting that the smaller mesh nets were almost as efficient as the larger nets at catching stage CIV copepodites.

There is some variability in the analyses of catch rate differences among cruises for most stages. For example, stage CII had one cruise in which the probability associated with rejecting the null hypothesis was 0.1906, compared with probabilities of 0.0065 and 0.0081 for the other two cruises. Similar results were obtained for other stages (Table 2). In each case the meta-analyses demonstrated, convincingly, that the overall result was highly in favour of rejecting the null hypothesis and that there was no inconsistency in these results. We explain the variability among cruises as being due to the skewed nature of the data where a few large catches occurred (Table 2).

Comparison of catch rates between the small bongos and the large bongos fitted with 0.505-mm-mesh nets demonstrated that stage CIII was undersampled by the larger bongos, while stages CV and CVI were undersampled by the small bongos (Table 4). As above, it appears that stages CI and CII were not staged for the 0.505-mm-mesh samples for this cruise. Therefore, there is no direct comparison available for these stages. However, given the highly significant differences for the 0.165- and 0.333-mm sample comparisons, we conclude that the same result applies here. There was no significant difference in catch rates for stage CIV at $P = 0.1588$. It might be argued that more samples may demonstrate a difference in catch rates for stage CIV based on comparisons presented in Table 2 where individual probability values higher than this were nevertheless considered to contribute to an overall conclusion of significant differences in catch rate between 0.165- and 0.333-mm-mesh samples.

Catch rate ratios, calculated as the mean of the ratios for each paired tow for each stage within each cruise, demonstrate the large variability observed in our samples (Table 5). For each stage this is evident in the variable ratios, the large coefficient of variation associated with each variable, and the often relatively large estimate of skewness. It is difficult to estimate a reliable catch rate ratio for any of the copepodite stages of *C. finmarchicus* based on these data.

Discussion

The variability associated with sampling zooplankton is well known. For most stages analyzed in this study there was a large degree of variability associated with comparison of catch rate differences among cruises. We observed no trends in seasonal differences associated with abundance or development that might explain the variability among cruises. Therefore, our analysis serves to point out that reliable estimates of catch rate differences are only obtained with a large number of samples. It also points out that deriving a simple ratio of catch rates would be an unreliable method for adjusting catch rates to a standard basis. Correction factors were calculated by Pillar (1984) for different mesh sizes used with four different plankton samplers. However, he cautioned against their use due to rapidly changing catch ratios with changing body length and "the inherent variability of a single (plankton) haul." Our catch ratios for paired tows varied widely with no apparent pattern in variability with respect to abundance or season. It is recommended, therefore, that different sized samplers be used simultaneously to sample different life history stages of *C. finmarchicus* and that simple correction factors not be applied.

The lower catch rates of younger copepodite stages by the large bongos (both 0.333- and 0.505-mm-mesh nets) would be due to a lower retention rate. On the other hand, the lower catch rates of the small bongos for later stage copepodites could have resulted from either avoidance or patchiness. Avoidance would occur due to a greater escape response by older, larger cope-

TABLE 4. Comparison of catch rate differences between 0.163- and 0.505-mm-mesh nets for different copepodite stages of *C. finmarchicus* sampled during one cruise (GAD050).

Stage	N	0.165-mm mesh Mean	0.165-mm mesh Skewness	0.505-mm mesh Mean	0.505-mm mesh Skewness	Wilcoxon T	Probability (one-sided)
CI	25	72.2044	3.528	0	—	0 (162)	$1/33554432 = 2.98 \times 10^{-8}$
CII	30	134.5281	2.961	0	—	0 (232)	$1/1.037 \times 10^{9} = 9.31 \times 10^{-10}$
CIII	31	161.1903	2.247	65.4732	2.749	87 (248)	0.0008
CIV	31	104.1408	1.410	104.9154	0.999	197 (248)	0.1588
CV	31	12.9313	1.488	15.6675	0.760	157 (248)	0.0373
CVI	31	11.8676	1.106	21.6021	1.796	62 (248)	0.00013

TABLE 5. Mean catch ratios and descriptive statistics calculated for each paired tow, for each stage within each cruise.

Stage	Cruise	*N*	Ratio	CV	Skewness
		(A) 0.165 to 0.333 catch ratios			
CI	ATC331	0	—	—	—
	GAD035	0	—	—	—
	GAD037	20	17.57	133.2	1.77
	GAD050	0	—	—	—
	GAD051	0	—	—	—
	HAWPAN	0	—	—	—
CII	ATC311	0	—	—	—
	GAD035	12	2.55	238.9	3.09
	GAD037	41	29.97	379.3	6.20
	GAD050	0	—	—	—
	GAD051	20	1.73	114.2	1.02
	HAWPAN	0	—	—	—
CIII	ATC331	20	2.66	277.8	4.13
	GAD035	24	1.01	238.8	2.92
	GAD037	41	1.25	119.8	2.22
	GAD050	9	2.07	90.5	0.19
	GAD051	40	1.02	57.5	0.09
	HAWPAN	27	0.54	176.4	2.11
CIV	ATC331	21	2.33	286.8	4.52
	GAD035	35	2.50	259.5	4.25
	GAD037	41	1.03	90.9	1.42
	GAD050	9	0.58	130.3	1.76
	GAD051	41	0.91	40.4	−0.35
	HAWPAN	39	0.86	75.8	0.42
CV	ATC331	21	2.56	257.0	4.54
	GAD035	35	1.86	212.4	3.34
	GAD037	41	0.92	123.7	2.32
	GAD050	9	0.51	159.3	2.64
	GAD051	41	0.94	54.7	2.17
	HAWPAN	40	1.14	112.2	4.91
CVI	ATC331	21	1.16	100.9	1.82
	GAD035	37	1.02	130.6	2.09
	GAD037	41	0.84	85.2	0.64
	GAD050	9	0.79	155.3	2.10
	GAD051	41	0.90	72.0	1.98
	HAWPAN	40	1.21	121.1	3.30
		(B) 0.165 to 0.505 catch ratios			
CI	GAD050	0	—	—	—
CII		0	—	—	—
CIII		30	3.85	142.0	2.79
CIV		31	0.94	73.4	0.91
CV		31	0.76	111.1	1.48
CVI		31	0.64	70.0	0.49

podites. McGowan and Fraundorf (1966) demonstrated that 20-cm ring nets caught fewer species of gastropods and euphausiids and had lower abundances than 60-cm ring nets. As mesh size and volume filtered were the same, they concluded that differences resulted from avoidance of the 20-cm sampler.

Patchiness provides an alternative explanation for the lower catch rates of CV and CVI stage copepodites. The large bongo is three times the diameter of the small bongo and, on average, filtered approximately 10 times as much water. Therefore, the small bongos might be expected to catch fewer copepods because of their patchy distribution, particularly at lower densities. For copepodite stage CV, average density varied among cruises by approximately 2.3 orders of magnitude, and CVI varied by approximately 1 order of magnitude. A simple ranking of higher catches between samplers for each stage indicates that differences in catch rates did not always disappear at higher densities (Table 2). The only clear exception was for stage CV at the highest densities observed (ATC331) where the small bongos caught more than the large bongos; there was no difference in catch rates at $P = 0.1526$. Therefore, this is one indication that differences in catch rates may, in part, have been due to patchiness and the smaller amount of water filtered by the small bongos. It suggests that if densities are high enough, or volume filtered is large enough, then catch rate differences for older copepodites would disappear.

We cannot distinguish between the effect of sampler size (diameter) and mesh size for lower catch rates of the older copepodite stages. Existing studies indicate that sampler size is more important (McGowan and Fraundorf 1966; Tremblay and Roff 1983). However, answering this question is of little relevance to studies using bongo samplers. A more important question concerns distinguishing between avoidance and patchiness as the reason for the observed lower catch rates of the small bongo. If catch rate differences between these samplers disappear when volume filtered by the small bongo equals that of the large bongo, then the difference would result from patchiness. In this case, researchers could use the small bongos to sample all copepodite stages of *C. finmarchicus*. On the other hand, this might result in long tow times to filter an equivalent volume of water and render such a sampling scheme impractical.

Acknowledgements

J. Runge and M. Paranjape reviewed an early version of this work and encouraged a more detailed analysis of the data, as presented here. We thank J. Hoenig for a thorough and constructive review of the present paper.

References

ANDERSON, J. T. 1990. Seasonal development of invertebrate zooplankton on Flemish Cap. Mar. Ecol. Prog. Ser. 67: 127–140.

ANDERSON, T. W., AND D. A. DARLING. 1952. Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. Ann. Math. Stat. 23: 193–212.

BAINBRIDGE, V., AND J. CORLETT. 1968. The zooplankton of the NORWESTLANT surveys. ICNAF Spec. Publ. No. 7: 101–122.

CONWAY, D. V. P., AND R. C. MINTON. 1975. Identification of the copepodid stages of some common calanoid copepods. Dep. Agric. Fish. Scot. Mar. Lab. New Ser. No. 7.

DAVIS, C. S. 1987. Predatory control of copepod seasonal cycle in the temperate ocean. J. Mar. Res. 45: 947–983.

EDGINGTON, E. S. 1972. An additive method for combining probability values from independent experiments. J. Psychol. 80: 351–363.

FISHER, R. A. 1932. Statistical methods for research workers. Oliver and Boyd, London.

GLOVER, R. S. 1967. The continuous plankton recorder survey of the North Atlantic. Symp. Zoo. Soc. Lond. 19: 189–210.

GLOVER, R. S., AND G. A. ROBINSON. 1968. Continuous plankton records during the NORWESTLANT surveys, 1963 – zooplankton. ICNAF Spec. Publ. No. 7: 123–126.

HEDGES, L. V., AND I. OLKIN. 1985. Statistical methods for meta-analysis. Academic Press, New York, NY.

HUNTLEY M., K. W. STRONG, AND A. T. DENGLER. 1983. Dynamics and community structure of zooplankton in the Davis Strait and Northern Labrador Sea. Arctic 36: 143–161.

KIMMERER, W. J. 1987. The theory of secondary production calculations for continuously reproducing population. Limnol. Oceanogr. 32: 1–13.

KRAUSS, M., AND J. TRAHMS. 1983. Zooplankton dynamics during FLEX '76, p. 632–661. *In* C. Sinderman and J. Lenz [ed.] North Sea dynamics. Springer-Verlag, Berlin.

McGowan, J. A., and V. J. Fraundorf. 1966. The relationship between size of net used and estimates of zooplankton diversity. Limnol. Oceanogr. 11: 456–469.

McLaren, I. A., J.-M. Sevigny, and C. J. Corkett. 1988. Body sizes, development rates and genome sizes among *Calanus* species. Hydrobiologia 167/168: 275–284.

McLaren, I. A., M. J. Tremblay, C. J. Corkett, and J. C. Roff. 1989. Copepod production on the Scotian Shelf based on life-history analyses and laboratory rearings. Can. J. Fish. Aquat. Sci. 46: 560–583.

Pillar, S. C. 1984. A comparison of the performance of fur zooplankton samplers. S. Afr. J. Mar. Sci. 2: 1–18.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, NY.

Smith, P. E., and S. L. Richardson. 1977. Standard techniques for pelagic fish egg and larval survey. FAO Fish. Tech. Pap. No. 175.

Tremblay, M. J., and J. C. Roff. 1983. Production estimates for Scotian Shelf copepods based on mass specific P/B ratios. Can. J. Fish. Aquat. Sci. 40: 749–753.

Williams, R., and J. A. Lindley. 1980. Plankton of the Fladen Ground during FLEX 76.I. Spring development of the plankton community. Mar. Biol. 57: 73–78.

Identification of *Thunnus* Tuna Species by the Polymerase Chain Reaction and Direct Sequence Analysis of their Mitochondrial Cytochrome *b* Genes

Sylvia E. Bartlett and William S. Davidson[1]

Department of Biochemistry, Memorial University, St. John's, Nfld. A1B 3X9, Canada

Bartlett, S. E., and W. S. Davidson. 1991. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome *b* genes. Can. J. Fish. Aquat. Sci. 48: 309–317.

Four commercially important tuna species in the genus *Thunnus* are caught off the east coast of Canada. The harvest of bluefin tuna (*T. thynnus*) is regulated, but that of bigeye (*T. obesus*), yellowfin (*T. albacares*), or albacore (*T. alalunga*) is not. Enforcement of the regulations governing the bluefin fishery has been difficult because of the close genetic relationships among these species and the ease with which morphological characters may be removed once a fish has been landed. Isoelectric-focusing of water-soluble muscle proteins does not resolve these four tuna species beyond two groups: one consisting of bluefin and yellowfin and the other comprising bigeye and albacore. The polymerase chain reaction was used to amplify a 307 base pair segment of the mitochondrial cytochrome *b* gene from members of these species. There is intraspecific variation at this locus in each of the species. More importantly, there are differences between the four species and these genetic markers can be used to determine the species identity of an individual tuna with a high degree of confidence. This methodology should prove very useful for enforcing the regulations governing Canada's bluefin tuna fishing industry.

Quatre espèces de thon du genre *Thunnus*, importantes du point de vue commercial, sont capturées au large de la côte est du Canada. L'exploitation du thon rouge (*T. thynnus*) est réglementée, mais celle du thon obèse (*T. obesus*), de l'albacore à nageoires jaunes (*T. albacares*) et du germon (*T. alalunga*) ne l'est pas. La mise en application du règlement régissant la pêche du thon rouge est difficile à cause de l'étroite relation génétique entre ces espèces et le fait que les structures morphologiques caractéristiques peuvent être facilement enlevées après la capture. L'électrofocalisation de protéines musculaires solubles dans l'eau ne sépare ces quatre espèces qu'en deux groupes, l'un composé du thon rouge et de l'albacore à nageoires jaunes et l'autre, du thon obèse et du germon. On a utilisé la réaction en chaîne déclenchée par la polymérase pour amplifier un segment de 307 paires de bases du gène mitochondrial du cytochrome *b* prélevé chez des échantillons de ces espèces. Il existe une variation intra-spécifique à ce locus chez chacune des espèces étudiées. Qui plus est, il existe des différences entre les quatre espèces et des marqueurs génétiques peuvent servir à identifier l'espèce à un niveau élevé de confiance. Cette méthode devrait se révéler utile dans la mise en application du règlement régissant l'industrie de la pêche du thon rouge au Canada.

Received March 19, 1990
Accepted November 23, 1990
(JA546)

Reçu le 19 mars 1990
Accepté le 23 novembre 1990

The bluefin tuna (*Thunnus thynnus*) is prized as one of the world's best marine sports fish and commands top prices in the lucrative Japanese *sashimi* market. The largest member of the family Scombridae, bluefin tuna is one of four species of *Thunnus* that are caught commercially off Canada's east coast. The other tuna species found in this region are bigeye (*T. obesus*), yellowfin (*T. albacares*), and albacore (*T. alalunga*). A fear that too many juvenile bluefin tuna were being caught brought about the formation of the International Commission for the Conservation of Atlantic Tunas (ICCAT) in 1969 and regulations governing the minimum size and limiting the total allowable catch of bluefin were introduced in the mid-1970s. The Canadian Department of Fisheries and Oceans has taken additional measures to maintain the bluefin tuna fishery within Canadian waters and these include restricting the number of licences issued, controlling the type of gear used, and limiting the number of bluefin tuna that may be taken per boat per day (Clay and Hurlbut 1988).

With the value of an individual bluefin tuna ranging from $1000 to $20 000 (1989 Canadian dollars) depending on the size and condition of the fish, it is not surprising that a black market in illegally caught bluefin tuna has arisen. This has made it difficult to maintain the quotas required to conserve and expand the bluefin tuna fishery. Enforcement has also been a problem because it is very difficult to tell a bluefin from a bigeye, yellowfin, or albacore (especially small fish), as all of these species are quite similar in appearance (Mather 1962). Moreover, the usual characters used to differentiate between these species are skeletal and visceral in nature (e.g. gillraker counts) (Mather 1962; Watson 1962; Gibbs and Collette 1966) and these identifying features are removed once the fish has been landed and gutted.

Biochemical studies have revealed close genetic relationships among species of *Thunnus*. Sharp and Pirages (1978) compared the electrophoretic mobilities of 13 enzymes in muscle extracts of tuna species. Phosphoglyceromutase and lactate dehydrogenase from *T. thynnus*, *T. obesus*, and *T. albacares*

[1]Author to whom correspondence should be addressed.

have identical mobilities that differ from the corresponding enzymes in *T. alalunga*. Fructose-1,6-diphosphatase from *T. obesus* migrates further upon electrophoresis than its counterparts in the other three species, and pyruvate kinase appears to give different patterns between each of the four species. In addition, glycerol-phosphate dehydrogenase from *T. thynnus* and *T. albacares* gave identical patterns which were different from those produced by the corresponding enzymes in *T. obesus* or *T. alalunga*. In conjunction with liver striation morphology, glycerolphosphate dehydrogenase was used in a biochemical identification of a bluefin tuna to establish a new California size record (Dotson and Graves 1984). Although allozyme electrophoretic patterns have proved very useful for identifying different tuna species, this procedure requires fresh tissue for optimum results. As this is rarely possible when one is dealing with seized tuna that is thought to have been caught illegally, this technique is not always readily applicable.

It is far preferable to examine DNA, the molecule of heredity, rather than proteins. DNA is the same in all cell types of an organism whereas proteins vary from tissue to tissue. DNA is remarkably stable and the amount of information available for analysis decreases as one goes from DNA to protein. This is because of the degeneracy of the genetic code and because protein electrophoresis only detects mutations that change the overall charge of the molecule. We have used a recent development in biotechnology, the polymerase chain reaction and direct nucleotide sequence analysis (Saiki et al. 1988; Kocher et al. 1989), to compare a segment of the mitochondrial cytochrome *b* genes of bluefin, albacore, bigeye, and yellowfin tunas. The nucleotide sequence of the cytochrome *b* gene can be used routinely and conveniently to determine the species identity of an individual tuna.

Materials and Methods

Source and Identification of Tuna

Samples of skeletal muscle were collected from the dorsal fin area of different tuna species by fisheries officers from Canada's Department of Fisheries and Oceans. The tuna were caught off southwest Nova Scotia. Samples of bluefin tuna from the Virgin Rock off Newfoundland were also examined. These samples were bagged individually, labelled, and either kept on ice or frozen at −20°C before being shipped in locked containers to the Biochemistry Department at Memorial University in St. John's. Full capture data including otolith number and a photo log of each sample were maintained. In addition, representative specimens of the four species of tuna examined in this study were delivered whole to the laboratory where the experiments were carried out. Identification of the representative specimens was confirmed using the keys in Scott and Scott (1988).

Electrophoretic Analysis of Muscle Proteins

Approximately 0.2 g of muscle tissue was homogenised by hand using a plastic pestle (Mandel Scientific) in a 1.5-mL Eppendorf tube in 1 mL of 12 mM Tris/borate buffer, pH 9.0. The homogenate was centrifuged at 12 000 × *g* for 15 min at 4°C. The supernatant was diluted 10-fold with sterile, deionised water and approximately 0.5 μL was applied to a Pharmacia isoelectric-focusing Phastgel™ system (pH range 3–9). After the isoelectric-focusing was completed, the gel was stained with Coomassie brilliant blue R350. Precast isoelectric-focusing gels and protein standards were obtained from Pharmacia (Canada) Ltd., Dorval, Que.

Extraction of DNA

It proved very difficult to obtain undegraded high molecular weight DNA from tuna muscle samples using the standard procedure of proteinase K digestion in the presence of SDS followed by phenol–chloroform extractions and ethanol precipitation (Maniatis et al. 1982). Three attempts to prepare mitochondrial DNA from tuna muscle by the method of Carr and Griffith (1987) failed. In our experience the method that routinely yielded tuna DNA of acceptable quality was a variation on the procedure of Chomczynski and Sacchi (1987). Briefly, 2–5 g of muscle was cut into small pieces and homogenised directly at room temperature with a glass-Teflon homogeniser in 10 mL of a solution containing 4 M guanidinium thiocyante, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. One and a half millilitres of 2 M sodium acetate, pH 4.1, was then added to the homogenate and the solution was mixed with 10 mL of water-saturated phenol and 4 mL of chloroform/isoamyl alcohol (24:1, v/v). After shaking, the mixture was left on ice for 15 min and then centrifuged at 10 000 × *g* for 20 min at 4°C. The aqueous phase was transferred to a fresh tube containing 10 mL of isopropanol. The solutions were mixed and left at −20°C for at least 1 h. Precipitated nucleic acids were obtained by centrifugation at 10 000 × *g* for 20 min at 4°C. The pellet was washed with cold (−20°C) 70% ethanol, dried briefly under vacuum, and dissolved in 10 mM Tris/HCl, 1 mM EDTA, pH 8.0, and stored at 4°C. This crude nucleic acid preparation was suitable for DNA amplification.

Amplification of the Mitochondrial Cytochrome *b* Gene

The procedure for amplifying the mitochondrial cytochrome *b* gene from a crude total cellular DNA preparation obtained from tuna muscle was adapted from the method of Kocher et al. (1989). The primers were designated cyt bH and cyt bL. The nucleotide sequence of cyt bH is 5′CCCCTCAGAATGATATTTGTCCTCA3′ and that of cyt bL 5′CCATCCAACATCTCAGCATGATGAAA3′. The primers were synthesized by W. S. Davidson in the Department of Biochemistry at Memorial University on a MilliGen/Biosearch cyclone DNA synthesiser in the DMT-off mode. It was not necessary to purify the oligonucleotides prior to use. Double-stranded amplifications were carried out in a final volume of 25 μL in 0.5-mL microcentrifuge tubes in a reaction cocktail containing 67 mM Tris/HCl, pH 8.8; 2 mM magnesium chloride; 10 mM 2-mercaptoethanol; 0.2 mM each of dATP, dCTP, dGTP, and dTTP; 10 pmol of each primer; and 1 unit of AmpliTaq DNA polymerase (Cetus, Emeryville, CA). A drop of mineral oil was added to cover the reaction mixture and 30 cycles of amplification (92°C for 45 s, 50°C for 45 s, and 72°C for 90 s) were carried out using a Perkin-Elmer (Irvine, CA) DNA Thermal Cycler. Fifteen microlitres of the reaction mixture was electrophoresed at 4°C through a 2% NuSieve (FMC BioProducts, Rockland, ME) agarose gel made up and run in a 40 mM Tris, 30 mM sodium acetate buffer titrated to pH 7.4 with HCl. The DNA was visualised by staining with ethidium bromide and the amplified fragment of DNA was excised from the gel and melted in 100 μL of sterile, deionised water. The sample was heated to 65°C for 5 min and mixed vigourously. Five microlitres of this solution was used to prepare a single-

stranded template by assymetric amplification (Gyllensten and Erlich 1988). The reaction was carried out in 100 μL of the same reaction cocktail and under the same cycling conditions as those used for the double-stranded amplification (see above) except that 40 pmol of the cyt bH primer was used and only 0.4 pmol of the cyt bL primer was present. Five microlitres of the reaction mixture was sufficient to visualise double- and single-stranded DNA bands following electrophoresis through a 2% agarose gel and staining with ethidium bromide. Residual nucleotides, primers, and buffer salts were removed from the single-stranded template by centrifugal dialysis using Centricon-30 filters (Amicon Ltd., Oakville, Ont.). Two millilitres of water was added to the reaction mixture and the solution was applied to the filtration apparatus and centrifuged at 3500 × *g* for 20 min at 20°C. An additional 2 mL of water was added and the centrifugation repeated. Another 2mL of water was added and the centrifugation was increased 1 h until the volume in the chamber was approximately 60 μL. Single-stranded DNA template was obtained in this final volume and 7 μL was taken for DNA sequencing.

Sequencing Reactions

Nucleotide sequencing of the single-stranded template was carried out using Sequenase and a commercial DNA sequencing kit purchased from United States Biochemical Corporation, Cleveland, Ohio. One picomole of cyt bL was used as the primer and α^{35}SdATP was used as the label. The sequencing reactions were performed according to the manufacturer's instructions except that the elongation mix was diluted 50-fold. The sequencing products were separated on 6% polyacrylamide denaturing gels (Sanger et al. 1977). The primers amplified a 307 base pair (excluding size of primers) segment of the cytochrome *b* gene (Kocher et al. 1989) and these procedures routinely allowed the sequence of 290 nucleotides of the noncoding strand to be determined.

Results

Comparison of Muscle Proteins

Isoelectric-focusing (pH range 3–9) of water-soluble muscle proteins from four tuna species yielded two groups: one characteristic of bluefin and yellowfin tunas and the other of albacore and bigeye (Fig. 1). However, it was not possible to discriminate between individual members within either group. Similar results were obtained using a narrower pH range (5–8).

Comparison of Mitochondrial Cytochrome *b* Gene Sequences

The nucleotide sequence of a portion of the complementary strand of the mitochondrial cytochrome *b* gene was obtained from 33 bluefin, 32 bigeye, 33 yellowfin, and 12 albacore tuna and this is illustrated in Fig. 2. A consensus sequence of this region was derived by comparing all of the sequences (Fig. 3). The numbering system is arbitrary but representative of results that could be obtained with ease from a single loading on a sequencing gel 40 cm in length. Number 296 identifies the nucleotide complementary to the first base of the codon for amino acid 33 (phenylalanine) of tuna cytochrome *b*.

The nucleotide sequences reveal intraspecific variation in this group of tuna (Fig. 3). For example, six genotypes were observed in the 33 bluefin that were examined. Twenty-eight of the bluefin tuna shared an identical genotype. Four of the other five genotypes differed by a single substitution from the consensus for this species, and the fifth had an additional nucleotide change. Four genotypes were observed among the 33 yellowfin examined. The most common genotype was shared by 28 fish. Three other yellowfin had a genotype that differed from the common type at two positions. Two additional yellowfin each had unique genotypes that differed from the common type by a single mutational event. The 12 albacore tuna gave four genotypes. Six albacore shared one genotype, four individuals shared another genotype, and two other genotypes were found in individual fish. These genotypes differ from the most common albacore sequence by one nucleotide substitution. Bigeye tuna showed the most intraspecific variation. Indeed, 11 different genotypes were detected among 32 bigeye tuna tested. There were two main groupings with nine individuals in each. Four bigeye shared a separate genotype as did two pairs. Another six individuals had unique genotypes. The extensive variation within this sample of bigeye suggests that there may be discrete breeding populations of bigeye tuna. The relationships of the genotypes to one another are complex and will be considered elsewhere (S. E. Bartlett and W. S. Davidson, in prep.).

Although intraspecific variation was observed at the cytochrome *b* locus in bluefin, bigeye, yellowfin, and albacore tuna, distinctive species-specific sequence differences were also uncovered in each of these four species of tuna. The data are summarised in Table 1. For example, the 32 bigeye tuna that were studied all had a thymine (T) at position 35 whereas members of the three other species had a guanine (G) (see Fig. 3). Similarly, the 33 yellowfin tuna had a cytosine (C) at position 62 whereas a T was present in the other species. Albacore tuna differed from all the other tuna at three sites (68, 89, and 227). The sample of 33 bluefin tuna had a C at position 260 whereas all other tuna examined had a T. These changes appear to be "fixed" in a particular species, thus providing the possibility of using them as genetic markers to define individual members of that species; but this is not the case. For example, the 12 albacore had an adenine (A) at position 29. All of the bluefin and yellowfin tuna had a G at this position as did 29 of 32 bigeye. However, three of the bigeye had an A at position 29. The most likely explanation is that one or more mutations have occurred at this position in the bigeye species. The pattern of nucleotide substitutions in mitochondrial cytochrome *b* genes predicts that this will be fairly common (Kocher et al. 1989; Irwin et al. 1991). A similar situation is seen at position 158 in bluefin tuna. All but one of the 33 bluefin have an A at this position whereas it is G in all members of the other species. Again, the most likely scenario to explain this observation is that a mutation has occurred at this site in the lineage leading to the variant bluefin. Position 113 provides yet another exam-

TABLE 1. Nucleotides at specific sites can be used to identify tuna species.

	Position					
Species	35	62	68	89	227	260
Bluefin	G	T	T	T	G	C
Bigeye	T	T	T	T	G	T
Yellowfin	G	C	T	T	G	T
Albacore	G	T	C/G	G	A	T

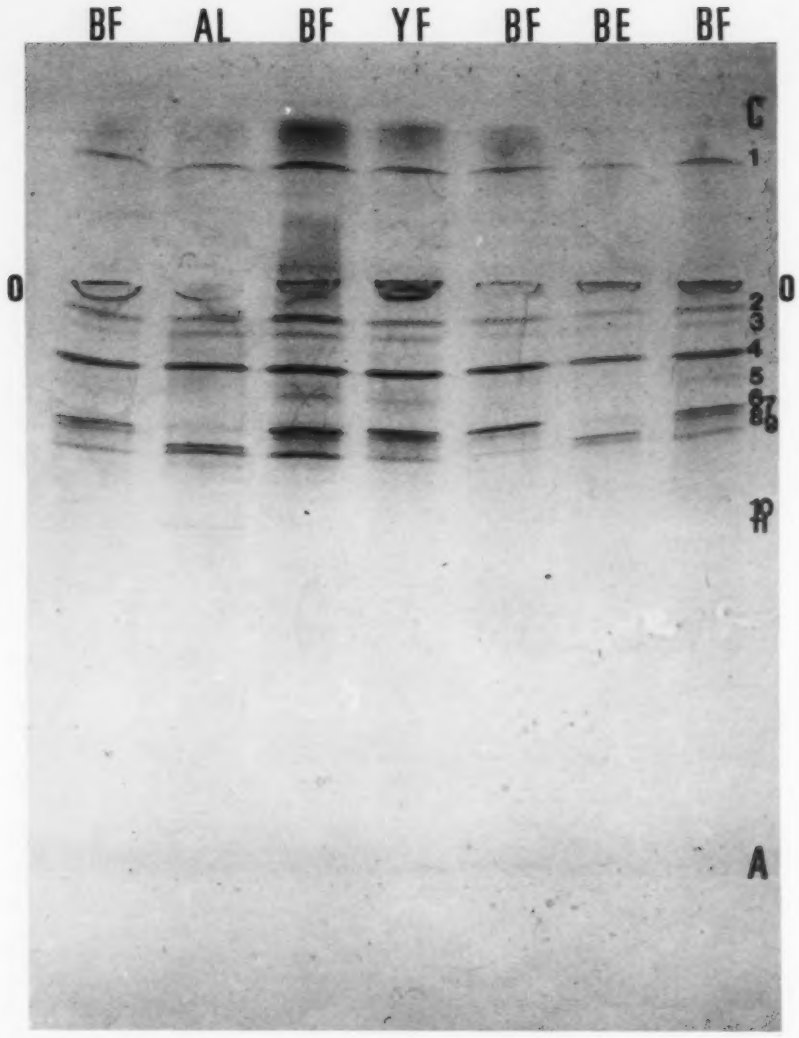


FIG. 1. Isoelectric-focusing gel (pH range 3–9) of water-soluble muscle proteins from different tuna. From left to right: lanes 1, 3, 5, and 7 are from bluefin (BF); 2 is from an albacore (AL); 4 is from a yellowfin (YF); and 6 is from a bigeye (BE). O represents the origin where the samples were applied. C and A indicate the cathode and anode, respectively.

ple of this phenomenon. The consensus sequence at this position was designated R for purine (A or G). Bluefin and bigeye have an A at this site but albacore and 30 of 33 yellowfin tuna had a G. A mutation from G to A in one lineage of yellowfin tuna could account for this change. Rather than using a particular nucleotide at a site to determine the species identity of an individual tuna, it is essential to use all the information available over the entire sequence. This is a form of pattern recognition and calls for a phylogenetic analysis. The nucleotide sequence information in Fig. 3 provides a frame work for this type of analysis. When the nucleotide sequence of the cytochrome *b* gene has been determined for a tuna of unknown species, the sequence can be fitted into the framework by using a computer program such as phylogenetic analysis using parsimony (PAUP) (Swofford 1989). The most likely species identity and an estimate of the reliability of the prediction can thus be calculated. This procedure also takes into account mutations in individual tuna that were not seen in this study.

None of the nucleotide substitutions resulted in a change in amino acid sequence. All but one of the substitutions are third position silent changes; the other detected in one of the bigeye is a first position C to T in a leucine codon and therefore is also silent. Most of the substitutions were transitions (i.e. the interchange of pyrimidines, C ↔ T, or purines, A ↔ G). However, we noted four instances of transversions (i.e. a change from a purine to a pyrimidine or vice versa) at positions 32, 35, 68, and 89. This latter type of nucleotide substitution accounted for approximately 20% of the changes observed among the tuna cytochrome *b* genes. This pattern of nucleotide substitution has been seen in other comparisons of fish cytochrome *b* genes (Kocher et al. 1989; Carr and Marshall 1991; McVeigh et al. 1991).

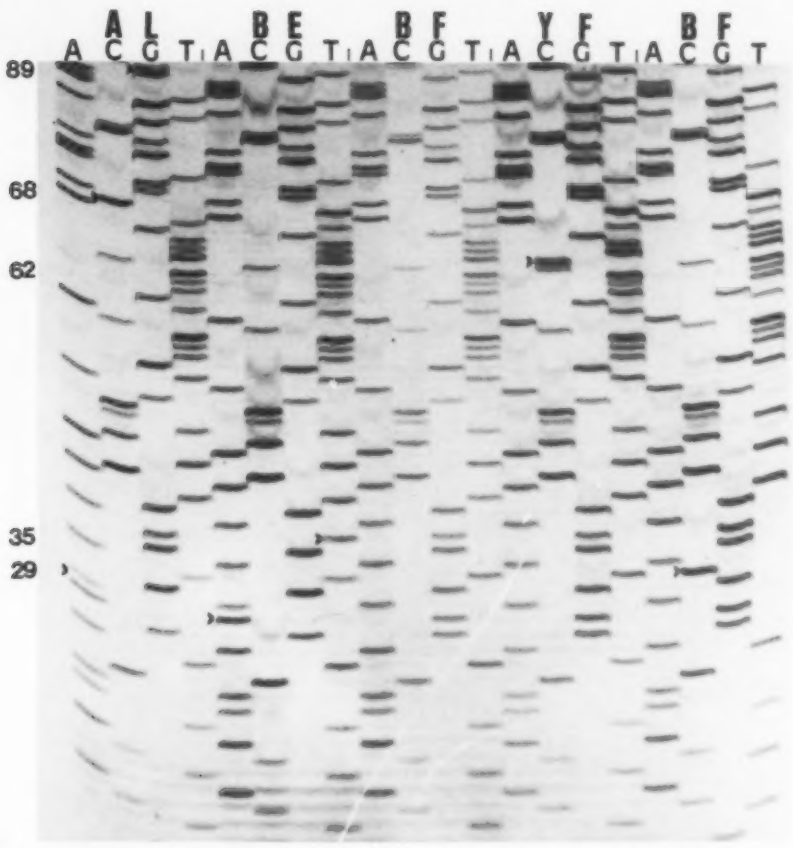


FIG. 2. Part of an autoradiograph of a DNA sequencing gel used to determine the sequence of the complementary strands of the cytochrome *b* genes of individual tuna: albacore (AL), bigeye (BE), bluefin (BF), and yellowfin (YF). The arrowheads show substitutions relative to the consensus sequence in Fig. 3. A, C, G, and T refer to adenine, cytosine, guanine, and thymine, respectively. The numbers at the left-hand side refer to positions in the consensus sequence given in Fig. 3.

Discussion

Whereas electrophoretic techniques are recommended for identifying the animal origin of meat, especially for fish species (AOAC 1984), even the sensitive procedure of isoelectric-focusing of water-soluble muscle proteins failed to identify individual *Thunnus* species. This confirmed that these species of tuna are genetically closely related (Sharp and Pirages 1978). Isoelectric-focusing could only separate the four species into two groups: one composed of bluefin and yellowfin and the other comprising bigeye and albacore.

Mitochondrial DNA restriction fragment length polymorphisms are capable of distinguishing between different species of Pacific salmon (Thomas et al. 1986) and this technique is potentially very useful for fisheries biologists (Gyllensten and Wilson 1987). However, the ability to employ this procedure on a routine basis for differentiating between bluefin, bigeye, yellowfin, and albacore tuna is severely curtailed because of the difficulty of obtaining intact mitochondrial or high molecular weight total cellular DNA. This is not a problem for the direct sequence analysis of fragments of DNA amplified via the polymerase chain reaction. A very small amount of DNA is required initially and the DNA does not have to be highly purified or of high molecular weight.

Kocher et al. (1989) showed that it was possible to design a pair of primers that correspond to highly conserved regions of the mitochondrial cytochrome *b* genes of vertebrates. These primers, in conjunction with the polymerase chain reaction, were used to amplify a 307 base pair segment of the mitochondrial genome and to determine the sequence of this gene from individual tuna. There are several advantages for using this mitochondrial gene for comparing closely related species (Kocher et al. 1989; Irwin et al. 1991). The amino acid sequences of cytochrome *b* from several diverse organisms have been determined and a consensus sequence showing invariant amino acid positions has been constructed. This serves as a guide to verify that the nucleotide sequence obtained in a forensic experiment is actually that of a cytochrome *b* gene. In addition, the pattern of nucleotide substitutions has been determined for many species of mammals, birds, and fishes. Within a particular species and also between closely related species, transitions are more common than transversions and most substitutions occur at synonomous sites such that the amino acid sequence of the protein is not altered.

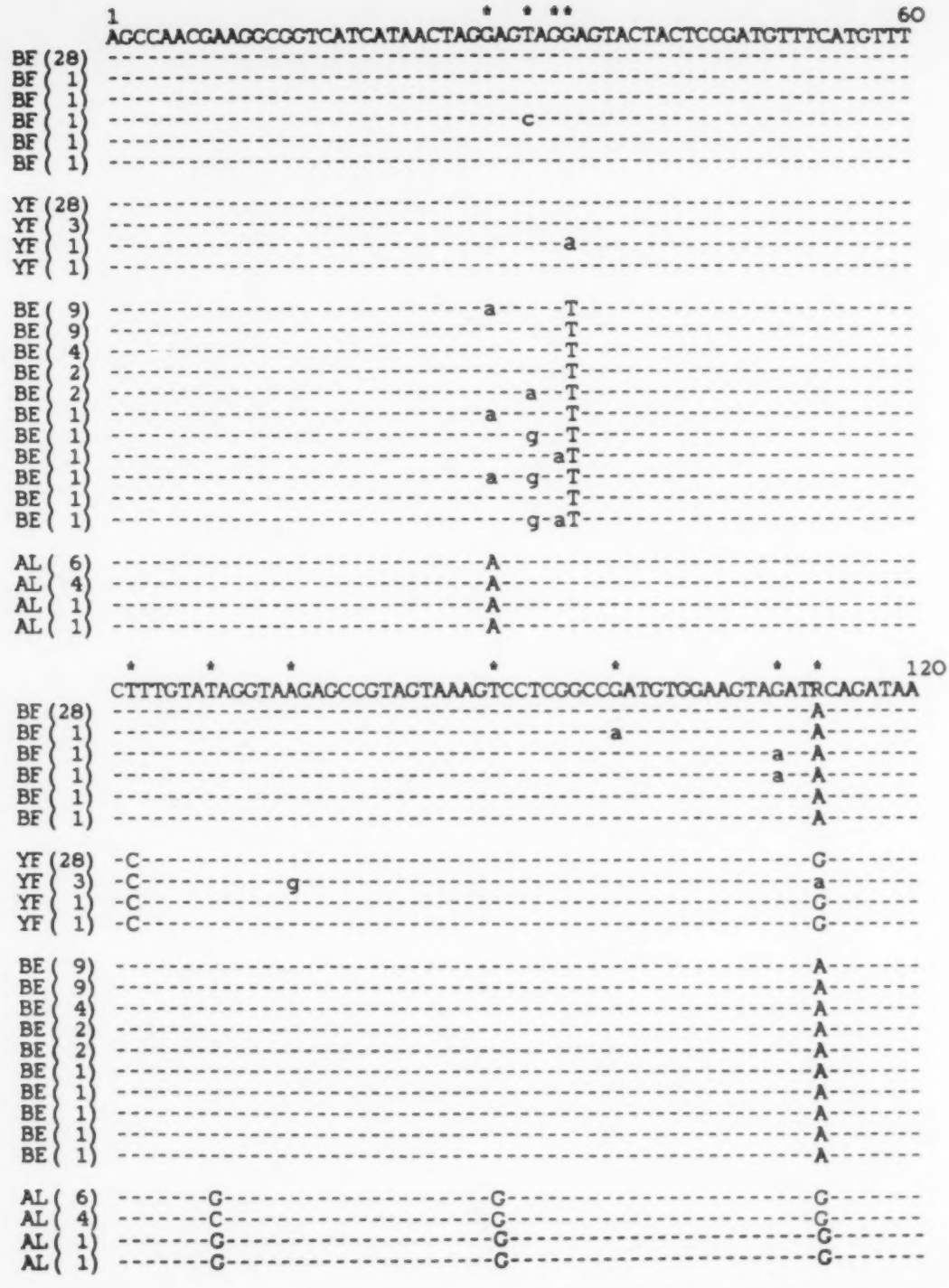


FIG. 3. Consensus sequence of part of the complementary strand of the cytochrome *b* genes of tuna in the genus *Thunus*: bluefin (BF), yellowfin (YF), bigeye (BE), and albacore (AL). Unless otherwise indicated, the sequence from an individual tuna is identical to that of the consensus. Intraspecific variation is indicated by small letters. Species-specific substitutions are shown by capital letters. The number in parentheses refers to the number of individuals of that species with that particular genotype. The final TTT in the sequence comes from one of the primers. (*Fig. 3 continued next page*)

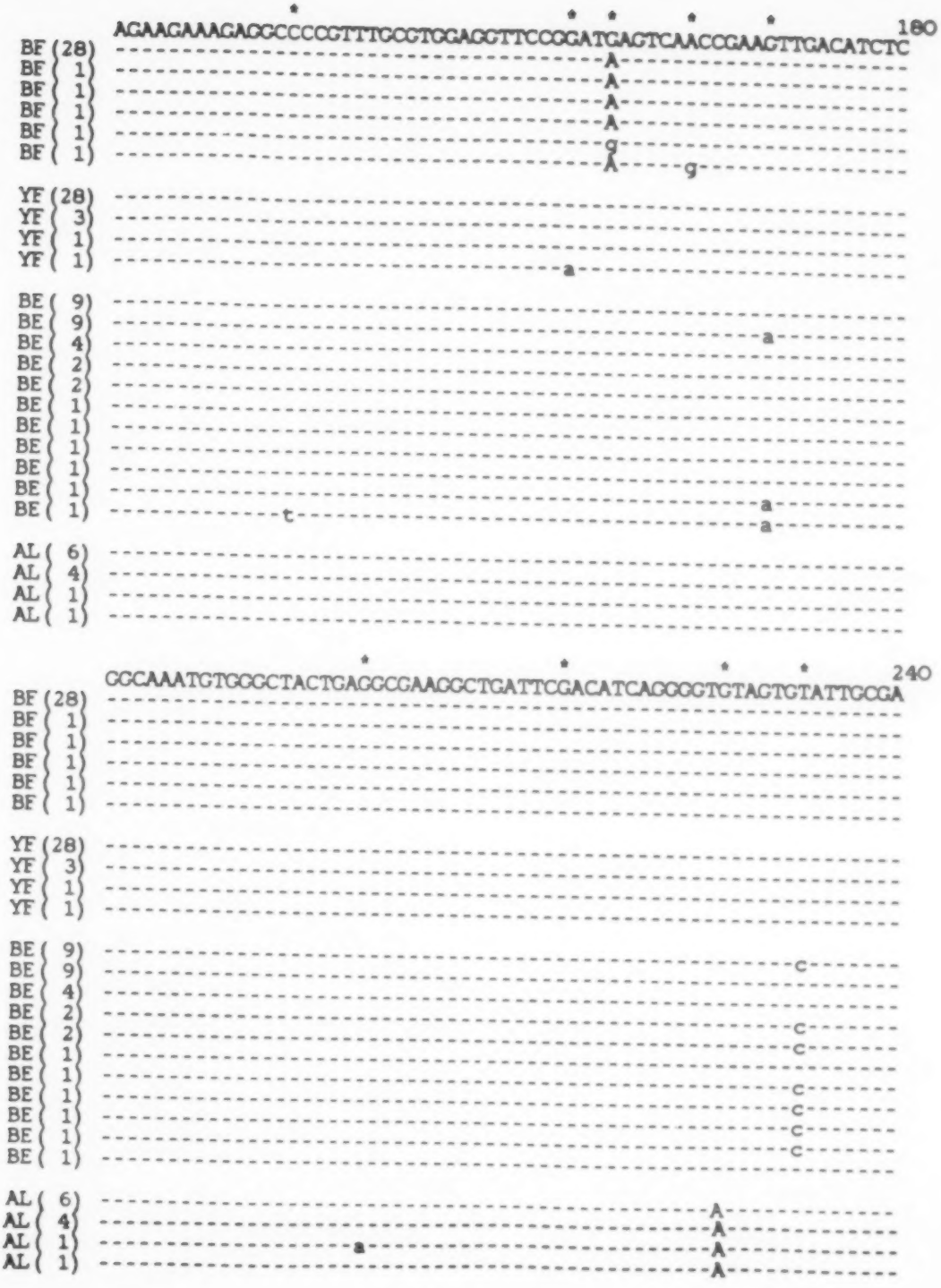


FIG. 3. *(Concluded next page)*

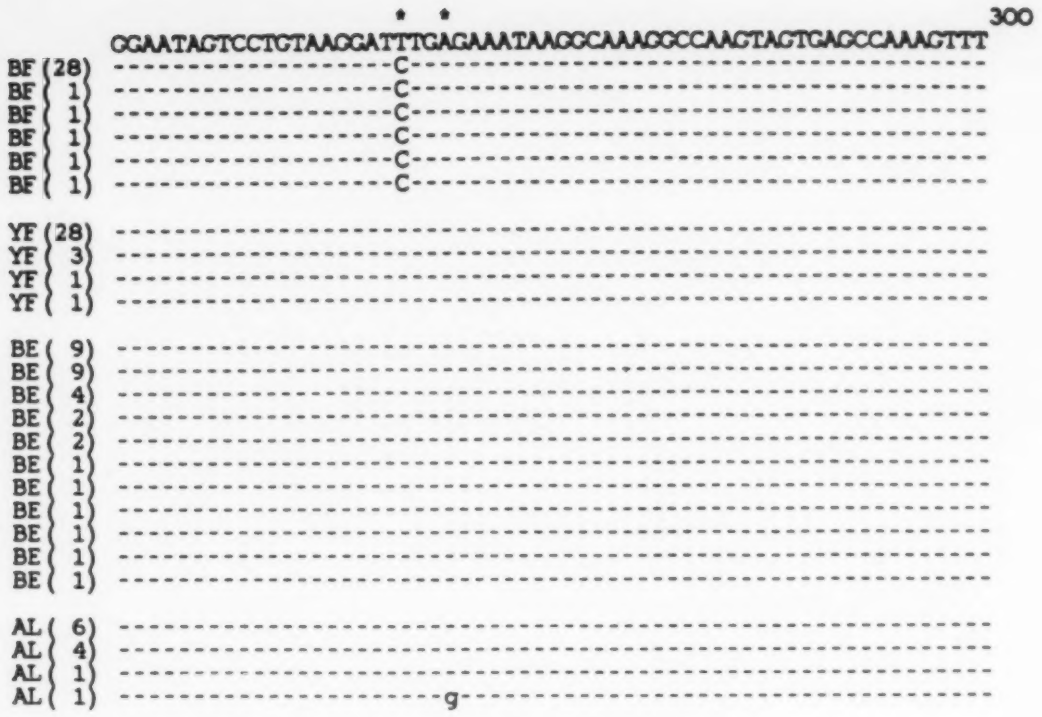


FIG. 3. (*Concluded*)

We therefore examined the DNA sequences of the cytochrome *b* genes of bluefin, bigeye, yellowfin, and albacore. The amount of intraspecific variation that was detected in these tuna, particularly bigeye, was quite startling and served to illustrate the resolving power of this procedure. For example, a search for restriction fragment length polymorphisms in mitochondrial DNA did not reveal any useful genetic markers that could discriminate between Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) (Graves et al. 1984). Similarly, an analysis of South African and California *T. alalunga* also failed to show population-specific markers (Graves and Dizon 1989). These results would suggest that little genetic variation exists within the latter tuna species. However, the nucleotide sequence data in this report may prompt a reexamination of these populations.

Although variation is apparent within the *Thunnus* tuna species, there are, more importantly, distinct differences which characterise each of the four species studied. These species-specific genetic markers make it possible to use this procedure and a phylogenetic analysis to determine unambiguously the species identity of an individual tuna. The methodology described herein should prove very useful for enforcing the regulations governing the bluefin tuna fishery off Canada's east coast. The methodology is also readily applicable to other enforcement and forensic problems.

Acknowledgements

We thank S. McQuaid, T. Owen, and K. Veinot for supplying samples and for their support and encouragement and R. Aubin, T. Birt, V. Birt-Friesen, B. Budowle, S. Carr, R. Fourney, J. Goodier, C. McGowan, and H. McVeigh for many helpful discussions. This research was supported financially by the Department of Fisheries and Oceans and the Centre for Fisheries Innovation.

References

AOAC. 1984. Official methods of analysis. 14th ed. 18.108–18.113. p. 349. Association of Official Analytical Chemists, Inc., Arlington, VA.

CARR, S. M., AND O. M. GRIFFITH. 1987. Rapid isolation of animal mitochondrial DNA in a small fixed-angle rotor at ultrahigh speed. Biochem. Genet. 25: 385–390.

CARR, S. M., AND H. D. MARSHALL. 1991. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome *b* gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Can. J. Fish. Aquat. Sci. 48: 48–52.

CHOMCZYNSKI, P., AND N. SACCHI. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate – phenol – chloroform extraction. Anal. Biochem. 162: 156–159.

CLAY, D., AND T. HURLBUT. 1988. Underwater World: bluefin tuna. Communications Directorate, Department of Fisheries and Oceans, Ottawa, Ont.

DOTSON, R. C., AND J. E. GRAVES. 1984. Biochemical identification of a bluefin tuna establishes a new California size record. Calif. Fish Game 70: 62–64.

GIBBS, R. H., AND B. B. COLLETTE. 1966. Comparative anatomy and systematics of the tunas, genus *Thunnus*. Fish Wildl. Ser. Fish. Bull. 66: 65–130.

GRAVES, J. E., AND A. E. DIZON. 1989. Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). Can. J. Fish. Aquat. Sci. 46: 870–783.

GRAVES, J. E., S. D. FERRIS, AND A. E. DIZON. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Mar. Biol. 79: 315–319.

GYLLENSTEN, U. B., AND H. ERLICH. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA–DQA locus. Proc. Natl. Acad. Sci. USA 85: 7652–7656.

GYLLENSTEN, U., AND A. C. WILSON. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes, p. 301–318. *In* N. Ryman and F. Utter [ed.] Population genetics and its application to fisheries management. University of Washington Press, Seattle, WA.

IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. (In press)

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86: 6196–6200.

MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Press, New York, NY.

MATHER, F. J. 1962. Tunas (genus *Thunnus*) of the western North Atlantic, p. 395–410. *In* Symposium on Scombroid Fishes. Part 1. Marine Biology Association of India, Mondapan Camp.

MCVEIGH, H. P., S. E. BARTLETT, AND W. S. DAVIDSON. 1991. Polymerase chain reaction/direct sequence analysis of the cytochrome *b* gene in *Salmo salar*. Aquaculture (In press)

SAIKI, R. K., D. H. GELFAND, S. STOFFEL, S. J. SCHARF, R. HIGUCHI, G. T. HORN, K. B. MULLIS, AND H. A. ERLICH. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science (Wash., DC) 239: 487–491.

SANGER, F., S. NICKLEN, AND A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74: 5463–5467.

SCOTT, W. B., AND M. G. SCOTT. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

SHARP, G. D., AND S. W. PIRAGES. 1978. Distribution of red and white swimming muscles, their biochemistry, and biochemical phylogeny of selected scombrid fishes, p. 41–78. *In* G. D. Sharp and A. E. Dizon [ed.] The physiological ecology of tunas. Academic Press, New York, NY.

SWOFFORD, D. L. 1989. PAUP: phylogenetic analysis using parsimony, Version 3.0b. Illinois Natural History Survey, Champaign, IL.

THOMAS, W. K., R. E. WITHLER, AND A. T. BECKENBACH. 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. Can. J. Zool. 64: 1058–1064.

WATSON, M. E. 1962. Tunas (genus *Thunnus*) of the western North Atlantic, p. 389–394. *In* Symposium on Scombroid Fishes. Part 1. Marine Biology Association of India, Mondapan Camp.

Modelling Bioaccumulation of Organic Pollutants in Fish with an Application to PCBs in Lake Ontario Salmonids

M. Craig Barber, Luis A. Suárez, and Ray R. Lassiter

Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, GA 30613, USA

Barber, M. C., L. A. Suárez, and R. R. Lassiter. 1991. Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Lake Ontario salmonids. Can. J. Fish. Aquat. Sci. 48: 318–337.

A model describing passive accumulation of organic chemicals from the aqueous environment and contaminated food in fish is developed. This model considers both biological attributes of the fish and physicochemical properties of the chemical that determine diffusive exchange across gill membranes and intestinal mucosa. Important biological characteristics addressed by the model are the fish's gill morphometry, feeding and growth rate, and fractional aqueous, lipid, and nonlipid organic composition. Relevant physicochemical properties are the chemical's molar volume and *n*-octanol/water partition coefficient (K_{ow}), which are used to estimate the chemical's aqueous diffusivity and partitioning to the fish's lipid and nonlipid organic fractions, respectively. The model is used to describe and to analyze the bioaccumulation of polychlorinated biphenyls (PCBs) in Lake Ontario alewife (*Alosa pseudoharengus*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and lake trout (*Salvelinus namaycush*).

Cet article présente un modèle dérivant l'accumulation passive dans les poissons de substances chimiques présentes dans l'eau ou des aliments contaminés. Ce modèle tient compte des caractéristiques biologiques des poissons de même que des propriétés physicochimiques de la substance qui déterminent sa capacité de diffusion à travers les membranes branchiales et la muqueuse intestinale. Les caractéristiques biologiques importantes prises en considération sont : la morphométrie des branchies, les taux de nutrition et de croissance et la composition organique fractionnaire des poissons (eau, lipides, substances non lipidiques). Les paramètres physicochimiques pertinents des substances considérées sont leur volume molaire et leur coefficient de partage *n*-octanol/eau (K_{ow}), lesquels sont utilisés pour estimer la diffusivité en milieu aqueux des substances et leur partage entre les fractions organiques lipidiques et non lipidiques, respectivement. Le modèle est utilisé ici pour décrire et analyser la bioaccumulation des biphényles polychlorés (BPC) dans le gaspareau (*Alosa pseudoharengus*), le saumon coho (*Oncorhynchus kisutch*), la truite arc-en-ciel (*Oncorhynchus mykiss*), la truite brune (*Salmo trutta*) et le touladi (*Salvelinus namaycush*).

Received November 23, 1988
Accepted August 14, 1990
(J9957)

Reçu le 23 novembre 1988
Accepté le 14 août 1990

When aquatic ecosystems are polluted with organic chemicals, resident fish will likewise become contaminated. If fish accumulate chemicals only from the water, this process is termed "bioconcentration" whereas if fish uptake chemicals from both water and food, the process is called "bioaccumulation." In either case, the internal accumulation of organic pollutants in fish constitutes an ecological threat not only to that species but also to its predators that include other fish, piscivorous birds and mammals, and man.

If organic pollutants are not metabolized, their ultimate concentrations in fish often can be predicted by assuming simple thermodynamic partitioning of the chemicals between the aqueous environment and the fish's hydrophobic components (primarily lipid). Such calculations, however, frequently fail to predict accurately field concentrations of extremely hydrophobic chemicals or of chemicals possessing high molecular volumes. Observed deviations can be in either direction, with calculated bioaccumulation factors being both considerably above and below those predicted by equilibrium partitioning. Either of these situations, however, can be explained and analyzed using process-based models that describe thermodynamically driven bioaccumulation of chemicals in fish.

In this paper, we describe the construction and parameterization of one such model, FGETS (Food and Gill Exchange of Toxic Substances), which simulates the bioaccumulation of nonmetabolized organic chemicals. This model is formulated such that its parameterization does not rely upon specific toxicokinetic studies but rather depends on physicochemical properties of the compounds of interest and on ecological, morphological, and physiological characteristics of the fish. After the model's presentation, we demonstrate its use by simulating and analyzing the bioaccumulation of polychlorinated biphenyls (PCBs) in Lake Ontario salmonids.

Model Formulation

Although whole-body concentration (micrograms per gram wet weight) of chemical pollutants in fish can be modelled using a single, aggregated differential equation (Thomann 1981; Thomann and Connolly 1984; Gobas et al. 1988; Thomann 1989), FGETS uses the approach of Norstrom et al. (1976) and Jensen et al. (1982) and generates these dynamics algebraically by simulating a fish's total body burden, B_f (micrograms), and body weight, W (grams wet weight), with the mass balance equations

$$(1) \quad \frac{dB_f}{dt} = J_g + J_i$$

TABLE 1. List of variables used in the text.

B_f	= total toxicant burden in whole fish (μg)
C_a	= toxicant concentration in aqueous fraction of the fish (μg/mL)
C_B	= bulk toxicant concentration of interlamellar water (μg/mL)
C_e	= toxicant concentration in egesta/feces (μg/mL)
C_f	= toxicant concentration in whole fish (μg/g (w))
C_{ia}	= toxicant concentration in aqueous fraction of intestinal contents (μg/mL)
C_{io}	= toxicant concentration in organic fraction of intestinal contents (μg/mL)
C_l	= toxicant concentration in lipid (μg/g (w))
C_o	= toxicant concentration in non lipid organic matter (μg/g (w))
C_p	= toxicant concentration in prey (μg/mL)
C_w	= toxicant concentration in environmental water (μg/mL)
d	= interlamellar distance (cm)
D	= diffusion coefficient in interlammelar water (cm^2/s)
E	= egestive flux (g (w)/d)
EX	= excretory flux (g (w)/d)
F	= feeding flux (g (w)/d)
h	= 0.5 d = hydraulic radius of interlamellar channels (cm)
I	= mass of food resident in the intestine (g (w))
J_g	= diffusive flux across the gills ($\mu g \cdot cm^{-2} \cdot s^{-1}$)
J_i	= diffusive flux across the intestine ($\mu g \cdot cm^{-2} \cdot s^{-1}$)
K_l	= partition coefficient between generic lipid and water (dimensionless)
K_e	= $(P_{ia} + P_{io} K_{oc})$ = partition coefficient for egested fecal matter (dimensionless)
K_f	= $(P_a + P_l K_l + P_o K_o)$ = thermodynamic bioconcentration factor (dimensionless)
K_{oc}	= partition coefficient between organic carbon and water (dimensionless)
K_{ow}	= partition coefficient between *n*-octanol and water (dimensionless)
K_{tw}	= partition coefficient between triolein and water (dimensionless)
K_o	= partition coefficient between nonlipid organic matter and water (dimensionless)
k_g	= conductance in interlamellar water (cm/s)
k_w	= permeability or conductance of lamellar wall (cm/s)
k_1	= uptake rate for first-order, linear kinetics (d^{-1})
k_2	= "depuration" rate for first-order, linear kinetics (d^{-1})
l	= lamellar length (cm)
N_{Gz}	= $(lD)/(h^2V)$ = lamellar Graetz number (dimensionless)
N_{Sh_w}	= wall Sherwood number (dimensionless)
P_a	= fraction of whole fish that is aqueous (dimensionless)
P_{ia}	= fraction of intestinal contents that is aqueous (dimensionless)
P_{io}	= fraction of intestinal contents that is organic (dimensionless)
P_l	= fraction of whole fish that is lipid (dimensionless)
P_o	= fraction of whole fish that is nonlipid organic matter (dimensionless)
Q	= ventilation volume (cm^3/s)
R	= routine respiratory flux (g (w)/d)
SDA	= respiratory flux due to specific dynamic action (g (w)/d)
S_g	= total gill surface area (cm^2)
V	= average velocity of interlamellar flow (cm/s)
W	= weight of fish (g (w))
X_g	= cross-sectional pore area of the gill (cm^2)
α_c	= assimilation efficiency of chemical (dimensionless)
α_f	= assimilation efficiency of food (dimensionless)
γ	= $W^{-1}dW/dt$ = fish's specific growth rate (d^{-1})
ΔP	= pressure differential across the gill sieve ($dynes/cm^2$)
η	= solution viscosity (poise)
ν	= molar volume (cm^3/mol)
ρ	= lamellar density (lamellae/mm)

$$(2)\quad \frac{dW}{dt} = F - E - R - EX - SDA$$

where J_g and J_i denote the net diffusive mass fluxes across a fish's gills from the water and across its intestine from food, respectively, and F, E, R, EX, and SDA are the fish's mass fluxes for feeding, egestion, routine respiration, excretion, and specific dynamic action (i.e. the additional respiratory expenditure in excess of R required to assimilate food), respectively. In the following three sections we will describe how each mass flux in equations 1 and 2 is formulated in FGETS. Table 1 provides the reader with a summarized list of the variables used in the development of these equations. In this presentation, the system of units used to describe chemical exchange is essentially the CGS system (centimetre, grams, second) whereas a CGD system (centimetre, gram, day) is used to describe fish growth. Therefore, the reader must remember that some unit interconversion is necessary to make the coupled system of equations dimensionally consistent. Interested readers can obtain a FORTRAN implementation of FGETS from the authors upon request.

Modelling Exchange from Water

Because the exchange of nonpolar organic chemicals across the gills of fish apparently occurs by simple diffusion, Fick's first law of diffusion can be used to model this process and, in particular, this exchange can be formulated as

$$(3) \quad J_g = S_g k_g (C_w - C_a)$$

where S_g is the fish's total gill area (square centimetres), k_g is the chemical's mass conductance (centimetres per second) across the gills from the interlamellar water, and C_w and C_a are the chemical's concentrations (micrograms per millilitre) in the environmental water and the fish's aqueous blood, respectively. Yalkowsky et al. (1973), Mackay (1982), Mackay and Hughes (1984), Gobas et al. (1986), and Gobas and Mackay (1987) used similar expressions of Fick's first law to describe chemical bioconcentration in fish.

To use this formulation in equation 1, a relationship between the fish's total body burden, B_f, and its aqueous blood concentration, C_a, must be specified. To this end, if a fish is conceptualized as consisting solely of water, lipid, and nonlipid organic matter, then its whole-body chemical concentration can be specified as

$$(4) \quad C_f = \frac{B_f}{W} = P_a C_a + P_l C_l + P_o C_o$$

where P_a, P_l, and P_o are the fractions of the whole fish that are water, lipid, and nonlipid organic material, respectively, and C_l and C_o are the chemical's concentrations in the fish's lipid and nonlipid organic matter, respectively. Because the depuration rates of organic chemicals from different tissues of a fish often do not differ significantly (Grzenda et al. 1970; van Veld et al. 1984; Branson et al. 1985; Norheim and Roald 1985; Kleeman et al. 1986a, 1986b), internal equilibration between these three phases can be assumed to be rapid in comparison with exchange across the gills. Consequently, equation 4 can be simplified to

$$(5) \quad C_f = (P_a + P_l K_l + P_o K_o) C_a$$

where K_l and K_o are thermodynamic partition coefficients between lipid and water and between organic carbon and water, respectively. Because at equilibrium, C_w equals C_a (see equation 3), this equation predicts that a fish's thermodynamic bioconcentration factor (i.e. the ratio, C_f/C_w, at equilibrium assuming only direct aqueous exchange) is

$$(6) \quad K_f = (P_a + P_l K_l + P_o K_o).$$

Combining equations 3, 5, and 6, the passive exchange of a chemical across a fish's gills now can be expressed as

$$(7) \quad J_g = S_g k_g \left(C_w - \frac{B_f}{W K_f} \right) = S_g k_g \left(C_w - \frac{C_f}{K_f} \right).$$

Although according to Fick's first law, the chemical conductance, k_g, could be specified as a ratio of chemical diffusivity to the thickness of an associated boundary layer, implementation of this definition is not straightforward. When diffusion occurs through a flowing medium, such as the interlamellar water, the thickness of the boundary layer varies spatially with the direction and velocity of flow. To resolve this problem, assume for the moment that a fish is only accumulating chemical and not excreting it appreciably, as might be the case in the initial phase of an exposure. In this case, equation 7 simplifies to

$$(8) \quad J_g = S_g k_g C_w .$$

Under the same conditions, however, the fish's branchial exchange also can be described by

$$(9) \quad J_g = Q (C_w - C_B) = Q \left(1 - \frac{C_B}{C_w} \right) C_w$$

where Q is the fish's ventilation volume (cubic centimetres per second) and C_B is the bulk concentration of the chemical in the water expired from the gills. To see the interrelationship among the parameters of equation 8 and 9, a fish's ventilation volume is expressed as the product of the gill's cross-sectional pore area, X_g (square centimetres), and the mean interlamellar flow velocity, V (centimetres per second). Because the gill's cross-sectional pore area is related to its lamellar surface area by

$$(10) \quad X_g = S_g \, dl^{-1}$$

where d (centimetres) is the mean interlamellar distance and l (centimetres) is the mean lamellar length (Hills and Hughes 1970), equation 9 can be written as

$$(11) \quad J_g = S_g \frac{dV}{l} \left(1 - \frac{C_B}{C_w} \right) C_w$$

and, therefore, the fish's uptake conductance can be specified formally as

$$(12) \quad k_g = \frac{dV}{l} \left(1 - \frac{C_B}{C_w} \right).$$

If certain assumptions are made concerning the geometry of the interlamellar spaces and the nature of mass transport between the secondary lamellae, functional expressions for both the mean interlamellar flow velocity and the normalized bulk water concentration (i.e. C_B/C_w) in equation 12 can be specified. In particular, because adjacent secondary lamellae form flat channels having very high aspect ratios (i.e. mean lamellar height/interlamellar distance), the secondary lamellae can be considered analogous to parallel plates and the flow of water between the lamellae can be treated as Poiseuille's slit flow (Hills and Hughes 1970; Stevens and Lightfoot 1986). In this case, the gill's mean interlamellar flow velocity can be quantified by

$$(13) \quad V = \frac{\Delta P \, h^2}{3 \eta \, l}$$

where ΔP is the pressure drop across the gill (i.e. approximately 250 dynes/cm^2 (Davis and Randall 1973)), h is the hydraulic radius of the channel, which equals one half the interlamellar distance (i.e. $h = 0.5d$), and η is the dynamic viscosity of water (i.e. 0.01 poise at 20°C).

The normalized bulk concentration of the exhalent gill water can be modelled using the solutions of the partial differential equations that describe steady-state convective mass transport

between parallel plates (see also Scheid and Piiper's (1971) Model IB for the analysis of gas equilibria across fish gills). In particular, again assuming fully developed Poiseuille's flow, chemical transport between secondary lamellae can be modelled as

$$(14)\quad \frac{3}{2}(1 - X^2)\frac{\partial \Theta}{\partial Y} = \frac{\partial^2 \Theta}{\partial X^2}$$

where Θ is the chemical's dimensionless concentration, X is the dimensionless distance normal to the surface of the lamellae such that $X = 0$ denotes the centerline of the lamellar channel, and Y is the dimensionless distance along the length of the secondary lamellae. Explicitly, these dimensionless variables are defined as

$$(15)\quad \Theta = \frac{C(x, y)}{C_w}$$

$$(16)\quad X = \frac{x}{h}$$

$$(17)\quad Y = \frac{y D}{h^2 V}$$

where $C(x, y)$ is the chemical's concentration between the lamellae at the distances x from the lamellar surface and y along the length of the lamellae and D (square centimetres per second) is the diffusivity of the chemical of interest. The dimensionless length or Graetz number, N_{Gz}, of the secondary lamellae is defined by equation 17 when $y = l$. Using this definition and the functional notation $\Theta(X, N_{Gz})$ to designate the dimensionless chemical concentration of water exhaled from the gill at the position X between adjacent secondary lamellae, the normalized bulk concentration, C_B/C_w, can be defined formally as the weighted average

$$(18)\quad \Theta_B = \frac{C_B}{C_w} = \frac{\int_0^1 \Theta(X, N_{Gz})(1 - X^2)\,dX}{\int_0^1 (1 - X^2)\,dX}.$$

That is, each concentration profile $\Theta(X, N_{Gz})$ is weighted by its relative velocity. Using the Graetz–Nusselt solutions reported by Brown (1960), Grimsrud and Babb (1966), Colton et al. (1971), and Walker and Davies (1974), this equation can be evaluated as

$$(19)\quad \Theta_B = \sum_{m=0}^{\infty} B_m \exp\left(-\frac{2}{3}\lambda_m^2 N_{Gz}\right)$$

where the coefficient B_m is determined by the mth eigenvalue, λ_m, and corresponding eigenfunction, f_m, of the ordinary differential equation

$$(20)\quad \frac{d^2 f}{dX^2} + \lambda^2 (1 - X^2) f = 0.$$

These eigenvalues and eigenfunctions are determined by the specific boundary condition that describe the rate of change of the chemical's concentration at the surface of the secondary lamellae. For nonreactive chemicals, the only mechanism that causes changes in concentration at the surface of the secondary lamellae is the flux of chemical across the gill epithelium. Assuming steady-state diffusion through both the interlamellar water and the gill epithelium, this boundary condition can be formulated as

$$(21)\quad \left.\frac{d\Theta}{dX}\right|_{X=1} = -N_{Sh_w}\Theta$$

where N_{Sh_w} is the channel wall Sherwood number, which is defined as

$$(22)\quad N_{Sh_w} = \frac{hk_w}{D}$$

where k_w is the permeability (or conductance) of the wall (see Grimsrud and Babb 1966 for details).

The important feature of equations 14–22 is that if wall Sherwood numbers for fish's gills can be specified, then analytical expressions for the bulk concentrations of chemicals in the exhalent gill water can be estimated theoretically. To determine representative wall Sherwood numbers for fish in general, we assumed that the permeability of the lamellar epithelium can be estimated simply by the ratio of a chemical's diffusivity through gill epithelium to the mean thickness of the gill's water-blood barrier. We further assumed that the diffusivity of gill epithelium is approximately one half the diffusivity of the interlamellar water as is the case for oxygen exchange across fish gills (Piiper et al. 1986) and the transport of molecules through fish cytosol (Sidell and Hazel 1987). After reviewing published morphometric data for the thickness of the gill's water-blood barrier (Hughes 1972; Hughes and Morgan 1973; Dube and Munshi 1974; Hughes et al. 1986) and interlamellar distances (Saunders 1962; Hughes 1966), we concluded that wall Sherwood numbers for fish gills can be assumed to equal approximately unity without loss of generality. In this case, equation 19 is approximated to greater than 99% accuracy using only the first eigenvalue (i.e. $\lambda_1 = 1$) of equation 20 (see Colton et al. 1971), and therefore, the bulk concentration of chemicals in a fish's exhalent gill water can be estimated simply by

$$(23)\quad \Theta_B = \frac{C_B}{C_w} = 0.99 \exp\left(-\frac{2}{3}N_{Gz}\right).$$

Besides determining the bulk concentration of the exhalent gill water, the lamellar Graetz number also influences the chemical conductance across fish gills in another way. If the right side of equation 12 is multiplied by $(hD)/(hD)$, terms can be rearranged to yield

$$(24)\quad k_g = \frac{D}{h}\left(\frac{1 - 0.99 \exp\left(-\frac{2}{3}N_{Gz}\right)}{\frac{1}{2}N_{Gz}}\right)$$

where the ratio D/h can be interpreted as the gill's minimum conductance assuming no interlamellar flow. In earlier work (Barber et al. 1988), we partitioned a fish's total exchange resistance between aqueous and tissue phases and estimated chemical conductance for gills by

$$(25)\quad k_g = \frac{D}{d}N_{Sh_f}$$

where N_{Sh_f} is an appropriately calculated mean fluid-side Sherwood number for the chemical transport to and from the

surface of the secondary lamellae. Equation 24, on the other hand, estimates a single, overall resistance for gill exchange. We believe that equation 24 is conceptually a better formulation.

Modelling Exchange from Food

Norstrom et al. (1976), Jensen et al. (1982), Thomann and Connolly (1984), and Niimi and Oliver (1987) modelled chemical uptake from food by assuming that a fish assimilates a constant fraction of the chemical it ingests, i.e.

$$(26)\quad J_i = \alpha_c C_p F$$

where α_c is an assimilation efficiency (dimensionless) for the chemical and C_p is the chemical's concentration (micrograms per gram wet weight) in the ingested prey. Because the exchange of nonpolar organic chemicals across the intestines of fish is driven by diffusive gradients (Vetter et al. 1985), however, this formulation would be thermodynamically based only if α_c is, in general, a decreasing function of the fish's total body concentration, C_f. To formulate J_i thermodynamically, we first considered the simple input-output relationship

$$(27)\quad J_i = C_p F - C_e E$$

where C_e is the chemical's concentration (micrograms per gram wet weight) in the fish's feces. Because the transit time of food through the gastrointestinal tract is relatively long, it is reasonable to assume that the concentrations of chemicals in the fish's aqueous blood, intestinal fluids, and dry fecal matter equilibrate with one another. Connell (1989) made a similar assumption to analyze the ratio of a predator's chemical concentration to that of its prey. This assumption implies that the chemical concentration in feces can be calculated in a manner similar to equation 5. In particular,

$$(28)\quad \begin{aligned} C_e &= (P_{ia} + P_{io} K_{oc}) C_{ia} \\ &= (P_{ia} + P_{io} K_{oc}) C_a \\ &= K_e \frac{C_f}{K_f} \end{aligned}$$

where P_{ia} and P_{io} are the fractions of the intestinal contents that are water and dry organic matter, respectively, C_{ia} is the concentration of the chemical in the aqueous portion of the intestinal contents, and K_{oc} is a thermodynamic partition coefficient between refractory organic matter and water (e.g. see Karickhoff 1981; Briggs 1981; Chiou et al. 1986).

If egestion is assumed to be a constant fraction of ingestion, i.e.

$$(29)\quad E = (1 - \alpha_f) F$$

where α_f is the fish's assimilation efficiency for food, then equations 27, 28 and 29 can be combined and factored to resemble equation 26. Doing so, one observes that

$$(30)\quad \alpha_c = 1 - (1 - \alpha_f) \frac{K_e C_f}{C_p K_f}.$$

Clearly, the dietary assimilation efficiency specified by this equation is a decreasing function of the fish's whole-body chemical concentration as suggested above. Although superficial inspection of equation 30 may suggest that a fish's dietary assimilation efficiency of a chemical is an increasing function of the chemical's concentration in its prey, this conclusion is naive because C_f and C_p are not independent once an exposure begins. In fact, as chemical concentrations in a fish's prey are increased, fish should accumulate whole-body concentrations more rapidly and, consequently, their mean observed assimilation efficiencies should decrease. Indeed, Opperhuizen and Schrap (1988) demonstrated that assimilation efficiencies of PCBs generally decrease with increasing prey concentrations.

TABLE 2. Summary of observed dietary assimilation efficiencies, α_c, of PCBs by coho salmon and rainbow trout calculated for various exposure intervals, (t_0, t_1). $B_f(t_0)$ and $B_f(t_1)$ identify the fish's whole-body burden at the start and end of the interval, respectively, and $I_c(t_0,t_1)$ denotes the total amount of PCB ingested by the fish during this time. Units of time are days, while units of body burden and total ingestion are μg/fish. Data for rainbow trout are as reported in table III of Lieb et al. (1974). Data for coho calculated from table I of Gruger et al. (1975) assuming fish body weight is described more accurately by the authors' reported regression, W (g) = 3.1 exp(0.007t (d)).

Chemical	t_0–t_1	$B_f(t_0)$	$B_f(t_1)$	$I_c(t_0,t_1)$	α_c
Aroclor 1254[a]	112–140	55	133	99	0.788
Aroclor 1254[a]	140–168	133	276	191	0.749
Aroclor 1254[a]	168–196	276	404	197	0.650
Aroclor 1254[a]	196–234	404	636	343	0.676
3,3′,4,4′-tetra-CB[b]	0–24	0	1.80	3.42	0.526
3,3′,4,4′-tetra-CB[b]	24–53	1.80	2.65	4.94	0.173
3,3′,4,4′-tetra-CB[b]	53–108	2.65	4.29	15.1	0.109
2,2′,4,4′,5,5′ = hexa-CB[b]	0–24	0	2.68	3.12	0.857
2,2′,4,4′,5,5′-hexa-CB[b]	24–53	2.68	4.18	4.51	0.333
2,2′,4,4′,5,5′-hexa-CB[b]	53–108	4.18	9.31	13.8	0.371
2,2′,4,4′,6,6′-hexa-CB[b]	0–24	0	2.42	3.32	0.729
2,2′,4,4′,6,6′-hexa-CB[b]	24–53	2.42	4.04	4.80	0.338
2,2′,4,4′,6, 6′-hexa-CB[b]	53–108	4.04	9.38	14.7	0.363

[a]Rainbow trout.
[b]Coho salmon.

Results of Lieb et al. (1974) and Gruger et al. (1975), who studied the dietary accumulation of PCBs in rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*), also corroborate our proposed fecal partitioning model. Data from these authors indicate that chemical assimilation efficiencies generally decrease as exposure time increases (see Table 2). To demonstrate that our fecal partitioning model generates similar dynamics, consider first the generalized bioaccumulation model

$$(31)\quad \begin{aligned} \frac{dC_f}{dt} &= (k_1 C_w + \phi C_p) - \left(\frac{k_1 + K_e \epsilon}{K_f} + \gamma\right) C_f \\ &= z - k_2 C_f \end{aligned}$$

where $k_1 = S_g k_g W^{-1}$, $\gamma = W^{-1}\, dW/dt$, $\phi = F/W$, and $\epsilon = E/W$ are the fish's specific rates of chemical uptake across the gill uptake, growth, ingestion, and egestion, respectively. This model is obtained by first substituting equations 7, 27, and 28 into equation 1 and then substituting equation 1 into

$$(32)\quad \frac{dC_f}{dt} = \frac{d(B_f/W)}{dt} = W^{-1} \frac{dB_f}{dt} - \gamma C_f.$$

Although k_1, γ, ϕ, and ϵ are in general time-varying coefficients because they depend on the fish's body weight, in laboratory studies for which growth during the experiment often is minimal, these coefficients can be treated as constants. In this case, the solution of equation 31 is

$$(33)\quad C_f = z\frac{(1 - \exp(-k_2 t))}{k_2}.$$

Substituting this expression into equation 30 and assuming only dietary exposure (i.e. $z = C_p\phi$), one obtains the following equation:

$$(34)\quad \alpha_c = \frac{k_1 + K_f\gamma + K_e\epsilon\exp(-k_2 t)}{k_1 + K_f\gamma + K_e\epsilon}.$$

That is, dietary assimilation efficiencies are predicted to decrease exponentially with time.

Modelling Bioenergetic Fluxes of Fish

In FGETS a fish's maximum, ad libitum consumption can be simulated using either a traditional temperature-dependent power function

$$(35)\quad F_{max} = \exp(f_0 (T - T_{opt}))\, f_1 W^{f_2}$$

where T_{opt} is the fish's optimal or preferred temperature (Paloheimo and Dickie 1965; Ursin 1967), or a process-oriented Rashevsky–Holling model that is defined by the equations

$$(36)\quad F_{max} = \phi (I_{max} - I)$$

$$(37)\quad \frac{dI}{dt} = F_{max} - A - E$$

where ϕ is the fish's ad libitum feeding rate (per day), I_{max} is the maximum amount of food that the fish can ingest (grams wet weight), I is the amount of food (grams wet weight) present in the intestinal tract, and A is the fish's assimilation (grams wet weight per day) (Rashevsky 1959; Holling 1966). Of course, simulating a fish's growth for long time intervals assuming continuous ad libitum consumption is ecologically unrealistic because prey availability usually limits feeding to submaximal levels. Therefore, a fish's realized feeding F, is formulated as

$$(38)\quad F = f(\cdot)\, F_{max}$$

where $f(\cdot)$ describes the fish's functional response to available prey. Presently, FGETS does not attempt to model the fish's functional response. Rather, $f(\cdot)$ can be used to calibrate a fish's predicted growth rate to field or observed growth rates.

A fish's assimilation and egestion is described simply as

$$(39)\quad A = \alpha_f D(I)$$

$$(40)\quad E = (1 - \alpha_f) D(I)$$

where α_f is the fish's assimilation efficiency, which is assumed to be a constant, and $D(I)$ is a function that describes the fish's pattern of intestinal evacuation. If equation 35 is used to describe a fish's ad libitum feeding, then FGETS assumes that $D(I)$ is identically equal to the fish's realized ingestion F (also see equation 29). However, when the Rashevsky–Holling model is used for this purpose, FGETS assumes that the fish's intestinal evacuation is described by the temperature-dependent power function

$$(41)\quad D(I) = \exp(\delta_0 (T - T_{opt}))\, \delta_1 I^{\delta_2}.$$

The numerical value of the exponent, δ_2, depends both on characteristics of the food item being consumed and on the mechanism that presumably controls gastrointestinal motility and digestion (Jobling 1981, 1987). For example, when gut clearance is controlled by intestinal peristalsis, δ_2 is assumed to equal 1/2, since peristalsis is stimulated by circumferential pressure exerted by the intestinal contents which, in turn, is proportional to the square root of its mass. On the other hand, when surface area is assumed to control the rate of digestion, δ_2 may be assumed to be either 2/3 or unity. If the fish (e.g. a piscivore) consumes a few large-sized prey, $\delta_2 = 2/3$ may be the appropriate surface area model whereas if the fish (e.g. a planktivore or drift feeder) consumes a large number of smaller, relatively uniform-sized prey, $\delta_2 = 1$ is appropriate because total surface area and total volume of prey becomes almost directly proportional to one another. Note that when δ_2 does equal unity, the Rashevsky–Holling model defined by equations 36, 37, 39, and 40 is analogous to the Elliott–Persson model for estimating daily rations of fish (Elliott and Persson 1978). Finally, Olson and Mullen (1986) outlined a hypothetical process-based model to explain even $\delta_2 = 0$.

It also should be noted that, in general, the coefficient δ_1 is not a simple constant but rather a power function of the fish's body weight, i.e.

$$(42)\quad \delta_1 = \delta_{1,1} W^{\delta_{1,2}}.$$

For example, Grove et al. (1978) showed that when an exponential model (i.e. $\delta_2 = 1.0$) is used to describe gastric evacuation of rainbow trout, emptying time was proportional to (fish weight).$^{0.7}$ Because this exponent is close to the theoretical value of 2/3 that would relate the trout's gut surface area to its body weight, this result could be explained by a surface area limitation for the absorption of digested food.

A fish's specific dynamic action, i.e. its respiratory expenditure associated with the digestion and assimilation of food, is modelled as a constant fraction of ingestion. In particular,

$$(43)\quad SDA = \sigma F$$

where $\sigma \approx 0.17$ (Ware 1975; Tandler and Beamish 1981; Beamish and MacMahon 1988).

To formulate expressions for body weight losses due to routine respiration and excretion, one must consider what are the primary end products of anabolic and catabolic metabolism that must be eliminated by a fish. In FGETS, we assume that ammonia, carbon dioxide, and water are the only waste products of fish metabolism (also see Machiels and Henken 1986, 1987). Because the elimination of metabolic water is insignificant with respect to a fish's total water balance (Loretz 1979), however, this flux is ignored in FGETS. Body weight loss due to respiration corresponds to a simple carbon flux which can be calculated from the fish's routine oxygen consumption, O_r (grams oxygen per day), using respiratory quotients, RQ (litres carbon dioxide respired per litre oxygen consumed), i.e.

$$(44)\quad R = \frac{12\text{ g (C)}}{\text{mole (CO}_2\text{)}} \cdot \frac{\text{mole (CO}_2\text{)}}{22.4\text{ L (CO}_2\text{)}} \cdot RQ \cdot \frac{22.4\text{ L (O}_2\text{)}}{\text{mole (O}_2\text{)}} \cdot \frac{\text{mole (O}_2\text{)}}{32\text{ g (O}_2\text{)}} \cdot O_r = \frac{12}{32} \cdot RQ \cdot O_r.$$

In FGETS, we assume that a fish's routine oxygen consumption is twice its basal or standard oxygen consumption (Ware 1975), which is described by the temperature-dependent power function

$$(45)\quad O_b = \exp(b_0 (T - T_{opt}))\, b_1 W^{b_2}.$$

Although ammonia excretion could be modelled using function analogous to equation 45 (e.g. see Paulson 1980), we have formulated this flux as a constant fraction of the fish's total respiration ($R + SDA$). Such a formulation seems reasonable because excretion and oxygen consumption generally track one another. For example, after feeding, ammonia excretion increases as does oxygen consumption (Brett and Zala 1975; Gallagher et al. 1984). Likewise, conditions that inhibit the passive excretion of ammonia also depress carbon dioxide excretion (Wright et al. 1989). FGETS assumes that fish maintain a constant nitrogen/carbon ratio, $NC = 0.25$ gN/gC, and estimates a fish's body weight loss due to excretion as

$$(46) \quad EX = \epsilon NC\,(R + SDA)$$

where $\epsilon = 17/14$ is the ratio of the molecular weight of ammonia to that of nitrogen.

If one knows a fish's specific growth rate, $\gamma = W^{-1}dW/dt$ (per day), and basal oxygen consumption, clearly all the mass fluxes in equation 2 can be back-calculated based on the preceding material (also see Thomann and Connolly 1984). We have constructed FGETS to facilitate this type of parameterization assuming that a fish's specific growth rate is described, in general, by

$$(47) \quad \gamma = \exp(\gamma_0\,(T - T_{opt}))\,\gamma_1\,W^{\gamma_2}.$$

Model Parameterization

Because reliable application of a model depends not only on the validity of its formulation but also on its parameterization, important aspects of parameterizing the above equations are now discussed.

Parameterizing K_f

Superficially, estimation of a fish's thermodynamic bioconcentration factor, K_f, via equation 6 appears to require a great deal of information. However, because the fish's body fractions, P_a, P_l, and P_o, are intercorrelated and because the partition coefficients, K_l and K_o, can be estimated using the *n*-octanol/water partition coefficient, K_{ow}, this important model parameter can be expressed as a function only of the fish's lipid fraction and K_{ow}.

Numerous studies (see Table 3) have shown that a fish's aqueous and lipid fractions are in general negatively correlated according to a simple linear function

$$(48) \quad P_a = a_0 - a_1\,P_l$$

where a_0 and a_1 are positive constants. Because the sum of P_a, P_l, and P_o must be unity, this relationship clearly necessitates that

$$(49) \quad P_o = (1 - a_0) - (1 - a_1)\,P_l.$$

To estimate a fish's K_f as a function of the fish's lipid content, FGETS assumes $a_0 = 0.82$ and $a_1 = 1.25$ (i.e. the means of the data summarized in Table 3).

K_{ow} has become the reference standard by which the thermodynamic partitioning of chemicals between the aqueous phase and virtually any organic phase is measured. To express K_f as a function of K_{ow}, one first must consider what are appropriate surrogates for the lipid and nonlipid organic components of whole fish. Because triglycerides are the principal storage lipid in fish, it seems reasonable to estimate K_l using a triglyceride/water partition coefficient. Assuming that triolein is an appropriate surrogate for fish triglycerides, least squares analysis of data from Patton et al. (1984), Chiou (1985), and D. Brockway (pers. com.) indicates that

$$(50) \quad K_l = K_{tw} = \begin{cases} 1.27\,K_{ow} & \text{if } K_{ow} < 10^5 \\ 213\,K_{ow}^{0.571} & \text{otherwise.} \end{cases}$$

To specify K_o, we assume that a fish's nonlipid organic matter is equivalent to organic carbon and use Karickhoff's (1981) regression between organic carbon/water partition coefficients, K_{oc}, and K_{ow}, to estimae this parameter. In particular,

$$(51) \quad K_o = K_{oc} = 0.411\,K_{ow}.$$

TABLE 3. Summary of regressions, $P_a = a_0 - a_1\,P_l$, between the aqueous and lipid fractions for fish.

Species	a_0	a_1	Source
Alosa caspia	0.79	0.78	Shubina and Rychagova 1981
Alosa pseudoharengus	0.81	1.04	Flath and Diana 1985
Catostomus sp.	0.81	1.35	Lowe et al. 1985
Coregonus sp.	0.79	0.73	Lowe et al. 1985
Cyprinus carpio	0.81	1.36	Lowe et al. 1985
Chrysophrys major	0.80	1.31	Morishita et al. 1987
Ictalurus sp.	0.80	0.94	Lowe et al. 1985
Ichthyomyzon gagei	0.89	1.26	Beamish and Legrow 1983
Micropterus sp.	0.80	1.77	Lowe et al. 1985
Oncorhynchus nerka	0.85	1.42	Brett et al. 1969; Groves 1970
Perca fluviatilis	0.79	1.11	Craig 1977
Stizostedion sp.	0.79	1.01	Lowe et al. 1985
Oncorhynchus mykiss	0.94	1.89	Weatherley and Gill 1983
Oncorhynchus mykiss	0.82	1.30	Staples and Nomura 1976
Salmo trutta	0.85	1.85	Elliott 1976
Salvelinus namaycush	0.79	1.34	Eschmeyer and Phillips 1965
Salvelinus n. siscowet	0.77	0.77	Eschmeyer and Phillips 1965
Sardines	0.81	1.01	Morishita et al. 1987
Sunfish[a]	0.81	1.80	Lowe et al. 1985
Trachurus japonicus	0.79	0.83	Kunisaki et al. 1986
Mean	0.82	1.25	

[a]*Lepomis* sp. and *Pomoxis* sp.

TABLE 4. Summary of allometric regressions, $S = s_1 W^{s_2}$ and $\rho = \rho_1 W^{\rho_2}$ where S is gill surface area (cm^2), ρ is lamellar density (lamellae·side^{-1}·mm^{-1}), and W is body weight (g (w)) for native and introduced North American freshwater fishes.

Species	s_1	s_2	ρ_1	ρ_2	Source
Catostomus commersoni	11.17	0.587	25.0	−0.107	Saunders 1962
Cyprinus carpio	8.46	0.794	32.2	−0.079	Oikawa and Itazawa 1985
Gambusia affinis	2.33	0.873	—	—	Murphy and Murphy 1971
Ictalurus nebulosus	4.98	0.728	15.8	−0.091	Saunders 1962
Lampetra planeri	25.5	0.616	28.8	−0.167	Lewis and Potter 1976
Lepisosteus sp.	3.94	0.738	38.8	−0.060	Landolt and Hill 1975
Micropterus dolomieui	7.31	0.820	30.0	−0.062	Price 1931
Oncorhynchus mykiss	1.84	1.125	—	—	Niimi and Morgan 1980
Oncorhynchus mykiss	3.14	0.932	27.5	−0.064	Hughes 1984
Stizostedion vitreum	0.80	1.129	—	—	Niimi and Morgan 1980
Tinca tinca	8.67	0.698	25.5	−0.030	Hughes 1972

Parameters for Gill Exchange

In general, a fish's total gill area is dependent on its body size according to the simple allometric function

$$(52) \quad S_g = s_1 W^{s_2}$$

and many authors have reported species-specific parameters for this function (see Table 4). In general, however, FGETS should be parameterized using the fish's effective gill surface area rather than its anatomical gill area (i.e. Table 4). In particular, because only 60 to 80% of the total lamellar surface area actually overlay capillaries (Byczkowska-Smyk 1957, 1958, 1959a, 1959b, 1961; Liszka 1969; Starmach 1971; Jakubowski 1977, 1982) and because only about 60% of a fish's secondary lamellae are routinely perfused (Booth 1978), the effective gill area of fish generally is only 36 to 80% of their anatomical gill area (Gehrke 1987). Even this estimate may be high because results of Paling (1968) suggest that not all lamellar channels are ventilated equally. FGETS uses a default correction of 1/2 to adjust anatomical gill area to effective gill areas.

To calculate a fish's lamellar Graetz number that determines its interlamellar chemical conductance, one must estimate both the mean interlamellar distance and length of the fish's secondary lamellae and the aqueous diffusivity of the chemical of interest. In general, each of these morphometric variables, like a fish's total gill surface area, depends on the fish's body size, i.e.

$$(53) \quad d = d_1 W^{d_2}$$

$$(54) \quad l = l_1 W^{l_2}.$$

Unfortunately, species-specific parameters for these allometric functions are seldom available. However, parameters for the former usually can be estimated using the interspecific regression

$$(55) \quad d = 0.118\rho^{-1.19} \; (n = 28; r = -0.92)$$

where ρ (lamellae per side per millimetre) is the density of lamellae on the gill's primary filaments which is itself an allometric function of the fish's body weight, i.e.

$$(56) \quad \rho = \rho_1 W^{\rho_2}.$$

Importantly, parameters for this function are generally available (see Table 4). To circumvent the paucity of morphometric relationships for lamellar lengths (for exceptions see Hughes 1984; Stevens and Lightfoot 1986), FGETS uses the interspecific regression

$$(57) \quad l = 0.0188 W^{0.294} \; (n = 90; r = 0.92).$$

Both equations 55 and 57 are functional regressions rather than simple linear regressions (see Rayner 1985; Jensen 1986) and the data used for their calculation were taken from Saunders (1962), Hughes (1966), Steen and Berg (1966), Muir and Brown (1971), Umezawa and Watanabe (1973), Galis and Barel (1980), and Hughes et al. (1986). In FGETS, a chemical's aqueous diffusivity, D (square centimetres per second), is estimated using the empirical relationship

$$(58) \quad D = 2.101 \times 10^{-7} \eta^{-1.4} \nu^{-0.589}$$

where ν (cubic centimetres per mole) is the chemical's molar volume (Hayduk and Laudie 1974).

Parameters for Food Exchange

To predict chemical exchange from food using the fecal partitioning model, the proximate composition of fish fecal material, i.e. the wet and dry fractions (P_{iw} and P_{id}) of a fish's intestinal contents, must be specified. Reported values for the percentage of moisture in the alimentary canal contents of fish vary between 50 and 80% (Brett 1971; Marais and Erasmus 1977; Grabner and Hofer 1985). More generally, however, it seems reasonable to assume that the water content of the intestine rapidly equilibrates with that of the fish's whole body, and therefore, P_{iw} essentially equals P_w. One implication of this assumption would be that meals with the same initial dry weight but different wet weights should be cleared from the intestine at the same rate because both meals should have essentially the same wet weight soon after ingestion due to their osmotic equilibration with the fish's whole body. Indeed, Garber (1983) found that initial dietary moisture content had no significant effect on the rate of gastric evacuation in yellow perch (*Perca flavescens*).

Parameters for Growth of Fish

Although the FGETS growth model has six individual mass fluxes, the only difficult tasks in parameterizing this model entail quantifying the fish's feeding, gastric evacuation, and respiration because the fish's assimilation, egestion, specific dynamic action, and excretion are formulated as linear combinations of these processes. However, because we have already discussed how respiration is estimated from oxygen consumption and what considerations should be made when selecting an appropriate gastric evacuation relationship, in this section we

will discuss how FGETS is parameterized to describe fish feeding.

If an allometric model is used to describe ad libitum feeding, the procedure to parameterize equation 2 is obvious. When a Rashevsky–Holling model is used to describe this process, the feeding rate, ϕ, can be estimated using the following equations:

$$(59)\quad M(t) = \int_0^t \phi\,(I_{max} - M(\tau))\,d\tau$$

$$(60)\quad \frac{dM(t)}{dt} = \phi\,(I_{max} - M(t))$$

$$(61)\quad -\phi t = \ln(1 - M(t)/I_{max})$$

where $M(t)$ denotes the total amount of food consumed during $(0, t)$ (also see Dunbrack 1988). If one knows the fish's gut capacity, I_{max}, and the meal size, M_{sat}, and time, t_{sat}, required to satiate fish, ϕ now can be readily calculated. In FGETS, we assume that $M_{sat} = 0.95\,I_{max}$ and therefore

$$(62)\quad \phi = -\frac{\ln(0.05)}{t_{sat}}.$$

Model Application

In this section, we will demonstrate FGETS by simulating the laboratory bioconcentration (i.e. gill uptake only) and field bioaccumulation (i.e. combined gill and food uptake) of PCBs in salmonids. This particular example application of FGETS was selected not only because of the environmental importance of these chemicals but also because some investigators have considered branchial uptake and excretion of extremely hydrophobic chemicals like PCBs to be insignificant (Weininger 1978; Thomann and Connolly 1984). If such assertions are indeed correct, then a detailed mechanistic description for a gill exchange model like that contained in FGETS might seem unwarranted.

Bioconcentration of PCBs by Laboratory Salmonids

Although bioconcentration of PCBs in fish has been studied by several authors under laboratory conditions, we selected the study of Oliver and Niimi (1985) to demonstrate the validity of the FGETS gill exchange algorithm. In this study, Oliver and Niimi investigated the bioconcentration dynamics of eight PCB isomers in rainbow trout exposed to environmentally realistic concentrations of PCBs. The initial body weight and mean lipid fraction of these fish were 180 g (w) and 0.073, respectively. To simulate the bioconcentration of PCBs in these fish, we parameterized FGETS using K_{ow}s reported in Hawker and Connell (1988) and the gill morphometric and physiological data summarized in Table 5. Because these fish were fed a daily ration equal to 2% of their body weight, however, the allometric model

$$(63)\quad F = 0.02W^{1.0}$$

was used to describe the fish's feeding for these simulations. Figures 1–3 display the observed and predicted bioconcentration dynamics of three of these PCBs, i.e. 2,5-dichlorobiphenyl, 2,2′,5-trichlorobiphenyl, and 2,2′,5,5′-tetrachlorobiphenyl. These results clearly indicate that FGETS can simulate the observed pattern of PCB bioconcentration in rainbow trout quite well.

Bioaccumulation of PCBs by Lake Ontario Salmonids

One of the most detailed studies on the patterns of PCB bioaccumulation in a natural ecosystem is that of Oliver and Niimi (1988), who determined concentrations of individual PCB isomers in coho salmon, rainbow trout, brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), alewife (*Alosa pseudoharengus*), which is the primary prey of these salmonids (Brandt 1986; Jude et al. 1987), zooplankton, and lake water of the Lake Ontario ecosystem during 1982 to 1984. To demonstrate FGETS using this data set, we simulated the bioaccumulation of tetra-, penta-, hexa-, and heptachlorobiphenyls in Lake Ontario salmonids for two different exposure scenarios. The first scenario, denoted Scenario I (C_w) where C_w is the water concentration used to parameterize the simulation, assumes that Lake Ontario salmonids feed only on alewife whose whole-body PCB concentrations are constant and equal to the values reported by Oliver and Niimi. This scenario implicitly assumes that alewife accumulate PCBs so rapidly that their whole-body concentrations are always at steady state with respect to their aqueous and dietary exposures of PCBs and therefore size-dependent predation by the salmonids of interest can be ignored when modelling their bioaccumulation of PCBs. The second scenario, denoted Scenario II (C_w), on the other hand, assumes that whole-body concentrations of PCBs in alewives are dynamic and that the size-dependent feeding relationships must be considered to model the bioaccumulation of PCBs in Lake Ontario salmonids. For this scenario, we assume that alewife feed on zooplankton/*Mysis*, which are thermodynamically equilibrated with the lake water, i.e.

$$(64)\quad C_z = P_{l_z} K_l C_w$$

where C_z and P_{l_z} denote the zooplankton's whole-body PCB concentration and lipid fraction, respectively (see Thomann and Connolly 1984; Connolly and Pedersen 1988) and that salmonids feed on alewife according to the relationship

$$(65)\quad L_a = 1.21 + 0.226\,L_s$$

where L_a (centimetres) denotes the mean length of alewives eaten by salmonids of length L_s (centimetres). This predator–prey relationship is simply the arithmetic means of the length–length regressions reported by Jude et al. (1987) for the salmonids of interest in Lake Michigan.

Because coho salmon, rainbow trout, brown trout, and lake trout have similar ecologies and physiologies, we treated these species as a single guild or compartment and simulated their growth using the Rashevsky–Holling feeding model. We parameterized FGETS generically for these fish using the geometric means of the species-specific data summarized in Table 5. Thus, parametric data described by power functions, $Y_i = a_i X^{b_i}$, were estimated as

$$(66)\quad Y = \sqrt[n]{\Pi\, Y_i} = \sqrt[n]{\Pi\, a_i}\, X^{\frac{1}{n}\sum b_i}.$$

Growth of alewife, on the other hand, was simulated using an allometric feeding model, and Table 6 summarizes the morphological and physiological data we used to parameterize FGETS for alewife.

To demonstrate any bioaccumulation model with a particular dataset, it is important that the data being simulated be as sympatric and temporally synchronous as possible. Therefore, because Oliver and Niimi (1988) collected salmonids and alewife sympatrically only in the Vineland Region of Lake Ontario

TABLE 5. Summary of physiological and morphological data used to parameterize FGETS for coho salmon, rainbow trout, brown trout, and lake trout. The indicated parameters represent the completed set of morphological and physiological data that must be input to FGETS when using a Rashevsky–Holling feeding model to simulate fish growth. Data are from (1) Beamish (1964), (2) Kutty (1968), (3) Carlander (1969), (4) Elliott (1975), (5) Muller-Feuga et al. (1978), (6) Grove et al. (1978), (7) Niimi and Morgan (1980), (8) Jensen et al. (1982), (9) Stewart et al. (1983), (10) Hughes (1984), (11) de la Noue and Choubert (1986), (12) Dunbrack (1988), and (13) Ruggerone (1989). All temperature dependent data are adjusted to 10°C by observed Q_{10}. W and L denote the salmonid's body weight (g (w)) and length (cm). Lipid fraction of Vineland salmonids is assumed to be $P_l = 0.09$ (Oliver and Niimi 1988).

Variable	Coho salmon	Rainbow trout	Brown trout	Lake trout
S_g (cm^2)	—	$2.40\ W^{1.03}$ (7,10)	—	—
ρ (lamellae/mm)	—	$27.5\ W^{-0.06}$ (10)	—	—
M_{sat} (g (w)/t_{sat})	$0.0893\ W^{0.88}$ (12)	$0.096\ W^{1.00}$ (6)	$0.046\ W^{0.76}$ (4)	—
t_{sat} (min)	—	$29.9\ W^{0.08}$ (6)	$1.28\ W^{0.73}$ (4)	—
α_f	—	$0.267\ W^{0.80}$ (5)	$0.139\ W^{0.86}$ (1)	$0.348\ W^{0.71}$ (9)
O (mg O_2/h)	—	0.75 (11)	—	0.74 (9)
D (g (w)/d)[a]	$1.75\sqrt{I}$ (13)	$1.93\sqrt{I}$ (6)	—	—
RQ	—	1.0 (2)	—	—
W	$0.0186\ L^{2.84}$ (8)	$0.0036\ L^{2.94}$ (3)	$0.025\ L^{2.86}$ (8)	$0.0054\ L^{3.12}$ (8)

[a]These are laboratory values and resulted in unexpectedly high growth rates when used unadjusted in FGETS. Because Boisclair and Leggett (1988) reported that field-determined gastric evacuation rates are approximately 0.4 to 0.8 that of laboratory rates for yellow perch, we adjusted these values by a factor of 0.8. This calibration yielded more realistic predicted growth dynamic and, therefore, was used in the simulations discussed herein.

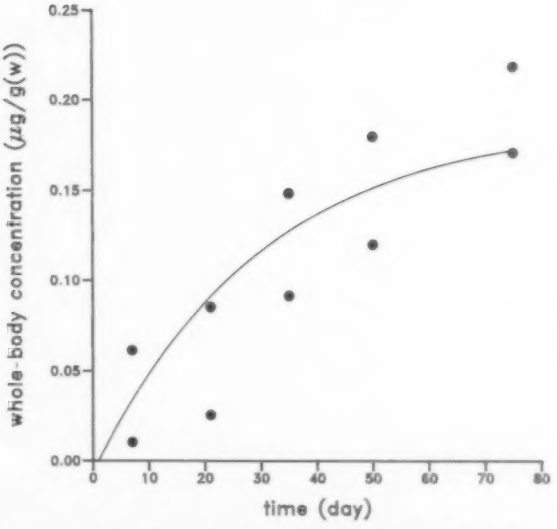


FIG. 1. Simulated bioconcentration of 2,5-dichlorobiphenyl in rainbow trout reported in Oliver and Niimi (1985). Circles denote the mean observed whole-body concentration plus or minus one standard deviation.

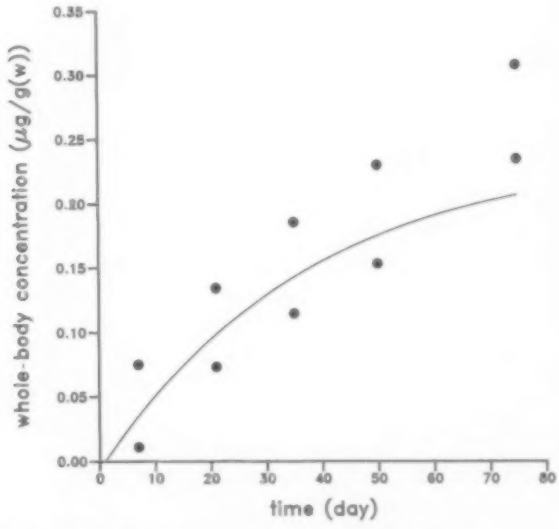


FIG. 2. Simulated bioconcentration of 2,2′,5-trichlorobiphenyl in rainbow trout reported in Oliver and Niimi (1985). Circles denote the mean observed whole-body concentration plus or minus one standard deviation.

during April to May 1982, we have restricted our demonstration of FGETS to this subset of data. Unfortunately, Oliver and Niimi did not measure aqueous PCB concentrations in connection with these Vineland collections. Consequently, we needed to consider whether their mean water concentrations for samples collected in April 1984 when Lake Ontario is well mixed were representative of the long-term aqueous exposures to which the fish collected at Vineland during April to May 1982 were subject. To make this evaluation, we compared the 1984 mean lakewide total PCB concentration with 1981 to 1983 total PCB concentrations in the Niagara River, which contributes not only 83% of Lake Ontario's total hydrologic input (Jaffe and Hites 1986) but also contributes directly to contaminant loading in the vicinity of Vineland (Simons et al. 1985). Based on this comparison (i.e. 1.1 ng/L in Lake Ontario and 8.6 ng/L in the Niagara River (B. G. Oliver, pers. com.)), we concluded that 1982 Vineland fish could have been exposed to aqueous concentrations much greater than the reported 1984 lakewide means.

Another line of evidence that suggests that the long-term Vineland aqueous exposures are underestimated by 1984 lakewide means is based on expected and observed suspended sediment/water partition coefficients, K_{ss}. In general, such partition coefficients are defined empirically as

(67) $K_{ss} = f_{oc} K_{oc}$

where f_{oc} is the organic fraction of the sediment and K_{oc} again is the organic carbon/water partition coefficient. Assuming the average organic fraction of Lake Ontario suspended sediments is 3.6% (Mackay 1989), using equation 51 one would anticipate that

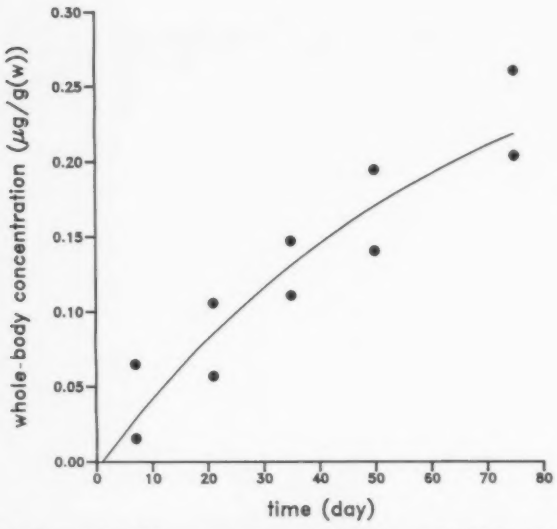


FIG. 3. Simulated bioconcentration of 2,2′,5,5′-tetrachlorbiphenyl in rainbow trout reported in Oliver and Niimi (1985). Circles denote the mean observed whole-body concentration plus or minus one standard deviation.

TABLE 6. Summary of data used to parameterize model simulations of the bioaccumulation of PCBs by alewife. All physiological data are adjusted to 10°C by observed Q_{10}. The indicated parameters represent the completed set of morphological and physiological data that must be input to FGETS when using the allometric feeding model to simulate fish growth.

Parameter	Function or value	Source
S_g (cm^2)	$36.5\ W^{0.930}$	See footnote a
ρ (lamellae/mm)	$35.4W^{-0.041}$	See footnote a
F_{max}	$0.0940\ W^{0.671}$	See footnote b
$f(\cdot)$	0.85	Assumed
α_f	0.84	Stewart and Binkowski 1986
O (mg O_2/h)	$1.46\ W^{0.785}$	Stewart and Binkowski 1986
RQ	1.0	Assumed
W	$0.0240\ L^{2.59}$	Brown 1972
P_l	$0.0167\ W^{0.413}$	See footnote c

[a]Interspecies regression for clupeids, *Alosa kessleri pontica* (Czolowska 1965), *Brevoortia tyrannus* (Hughes 1966), *Clupea harengus* (Hughes 1966; Steen and Berg 1966), and *Hilsa ilisha* (Pandey et al. 1987).

[b]Calculated as the product of Lake Ontario's nominal concentration of grazeable zooplankton, 1.40×10^{-3} g (w)/L (Scavia 1980; Flint 1986), and the daily filtering rate, $67.3W^{0.671}$ L/d, of gizzard shad (*Dorosoma cepedianum*) (Drenner et al. 1982), which was corrected to 10°C assuming a $Q_{10} = 2$.

[c]Regression analysis of data digitized from Flath and Diana (1985) yielded $P_l = 0.0277\ W^{0.413}$. This allometric function subsequently was adjusted so that a 32-g (w) alewife has a lipid fraction equal to 0.07 (see Oliver and Niimi 1988).

(68) $K_{ss} = 0.0144\ K_{ow}$.

Using Oliver and Niimi's (1988) data for tetra-, penta-, hexa- and hepta- isomers, we calculated observed suspended sediment/water partition coefficients

(69) $$\hat{K}_{ss} = \frac{C_{ss}}{C_{w,a}}$$

where C_{ss} is the isomer's measured concentration (nanograms per gram dry weight) in suspended sediments and $C_{w,a}$ is the isomer's adjusted aqueous concentration (nanograms per millilitre) calculated by equation 72 (see following discussion). When we calculated a functional regression between these partition coefficients, the relationship

(70) $\log \hat{K}_{ss} = 0.880 \log K_{ow} + 0.134$ ($n = 38$; $r = 0.64$)

was obtained. The slope of this regression is not significantly different from unity and therefore can be rewritten as

(71) $\hat{K}_{ss} = 0.229\ K_{ow}$.

Simple comparison of equations 68 and 71 indicate that the observed suspended sediment/water partition coefficients are approximately 15 times higher than the expected values. Although some of this difference certainly can be attributed to variations in the sediment's organic carbon fraction and to the particular K_{oc}/K_{ow} relationship used, much of this discrepancy can be explained by underestimated water concentrations.

TABLE 7. Summary of physicochemical data used to parameterize model simulations of the bioaccumulation PCBs by alewife and salmonids in Lake Ontario. Measured aqueous concentrations, $C_{w,m}$, as reported by Oliver and Niimi (1988); "dissolved" aqueous concentrations, $C_{w,a}$, were calculated as discussed by Chiou et al. (1986) assuming doc is 2 mg/L.

Homolog group	$C_{w,m}$ (pg/L)	$C_{w,a}$ (pg/L)	log K_{ow}[a]	v[b]
tetra-CBs	300	285	5.91	210
penta-CBs	390	356	6.34	228
hexa-CBs	160	130	6.75	245
hepta-CBs	54	29	7.19	263

[a]See Hawker and Connell (1988).

[b]Molar volume (cm^3/mol) calculated by the Le Bas method.

Based on these considerations, we parameterized both Scenarios I and II using the 1984 lakewide means and water concentrations 7.5 times greater than these values, i.e. concentrations representative of the Niagara River during 1981 to 1983. Oliver and Niimi (1988) noted that as much as 50% of their measured water concentrations could be bound to dissolved organic carbon (doc). Because such chemical may not be available for gill exchange (Black and McCarthy 1988; Servos et al. 1989), water concentrations used to parameterize Scenarios I and II were adjusted accordingly. We calculated "actual" dissolved water concentrations ($C_{w,a}$) from "measured" values ($C_{w,m}$) using the formula

(72) $$C_{w,a} = \frac{C_{w,m}}{1 + C_{doc} K_{doc}}$$

where $C_{doc} = 2 \times 10^{-6}$ g/mL and $K_{doc} = 0.0068\ K_{ow}^{1.07}$ (see Chiou et al. 1986; Servos et al. 1989). For these calculations, we used K_{ow}s reported by Hawker and Connell (1988). Table 7 summarizes Oliver and Niimi's (1988) measured water concentrations, adjusted water concentrations calculated by equa-

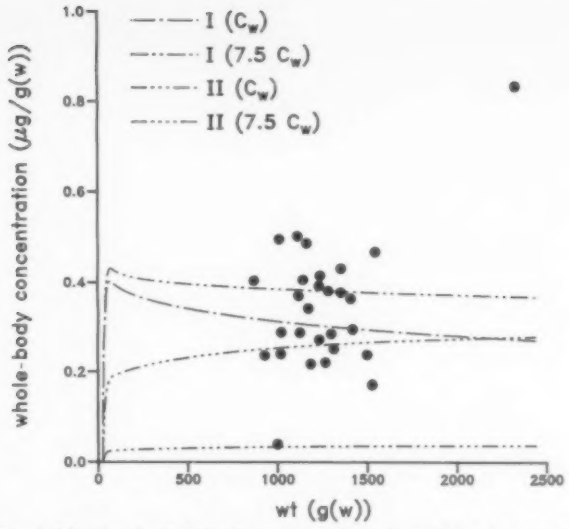


FIG. 4. Simulated whole-body concentration of tetrachlorobiphenyls in Lake Ontario salmonids plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

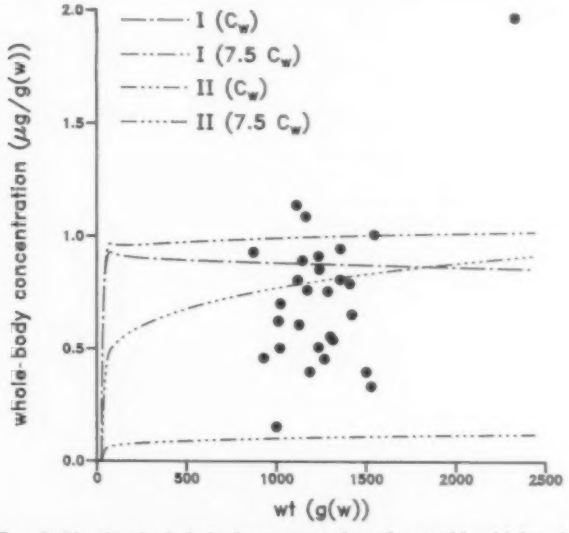


FIG. 5. Simulated whole-body concentration of pentachlorobiphenyls in Lake Ontario salmonids plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

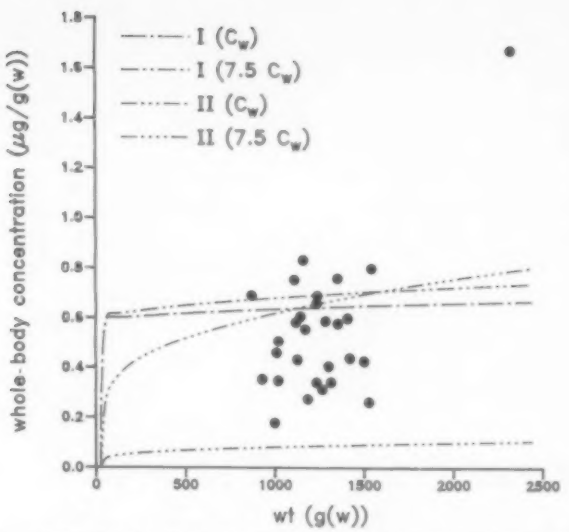


FIG. 6. Simulated whole-body concentration of hexachlorobiphenyls in Lake Ontario salmonids plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

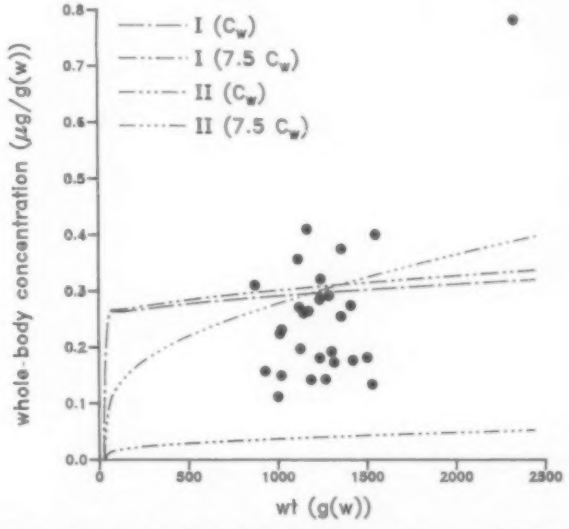


FIG. 7. Simulated whole-body concentration of heptachlorobiphenyls in Lake Ontario salmonids plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

tion 72, and the physicochemical properties of PCB homologs used in the simulations discussed below.

Figures 4–7 display observed and predicted whole-body concentrations of PCBs in Lake Ontario salmonids as a function of their body weight. With the obvious exception of Scenario II (C_w), each simulated trajectory passes through the bulk of observed data. The fact that these simulations were obtained without site-specific calibration and that most of the observed whole-body concentrations of tetra-, penta-, hexa-, and hepta-CB in these fish vary only by a factor of 2 to 4 is a strong indication of the validity of FGETS. Although one still might argue that the scatter in the observed data makes it difficult to judge how well FGETS actually describes PCB bioaccumulation in Lake Ontario salmonids, much of variation in the observed data can be attributed to variations in the fish's lipid fraction (i.e. $0.04 < P_l < 0.17$), which is assumed to be constant in our simulations.

Although biomagnification factors, *BMF*, are sometimes defined as the ratio of a predator's whole-body chemical concentration to that of its prey, these values also are defined as the factor by which bioaccumulation of chemicals due to joint aqueous and food chain exposure exceeds the bioconcentration of chemicals expected assuming only aqueous exchange. Because $C_w K_f$ is the expected equilibrium concentration of a chemical in a fish that accumulates chemical only from the water (see equations 5 and 6), a fish's biomagnification factor can be defined as

$$(73)\quad BMF = \frac{C_f}{C_w K_f} = \frac{BAF}{K_f}$$

where C_f is the fish's whole-body chemical concentration measured under field conditions or predicted by model simulations assuming joint food and water exposure and the ratio $C_f/C_w = BAF$ is the fish's bioaccumulation factor. Because branchial exchange of chemicals is conceptually analogous to thermodynamic partitioning of chemicals between two phases, a fish's biomagnification factor defines the degree to which fish have accumulated chemical over and above that which might be predicted by thermodynamic partitioning. Clearly, because whole-body concentrations of chemicals in fish are generally time varying, their calculated biomagnification factors will also be time varying. Although, for Scenario I (C_w), the final predicted salmonid *BMF*s are 11, 13, 13, and 11 for tetra-, penta-, hexa-, and hepta-CB, respectively, these values decrease as water concentrations increase. Thus, for Scenario I (7.5 C_w), final *BMF*s of Lake Ontario salmonids are calculated to be 2.0, 2.1, 1.9, and 1.6 for tetra-, penta-, hexa-, and hepta-CB, respectively. For Scenario II the predicted salmonid *BMF*s are 1.6, 1.9, 2.1, and 1.9 for tetra-, penta-, hexa-, and hepta-CB, respectively, regardless of the assumed aqueous concentrations of PCBs.

Because the chemical exchanges across a fish's gills and intestine are formulated explicitly and implicitly as processes driven by chemical/thermodynamic gradients, one might wonder how FGETS predicts any degree of biomagnification. The answer to this question depends simply on the fact that thermodynamically driven mass exchange is a function of the intensive property of chemical activity or concentration. As food is assimilated, the concentration of chemical remaining in the intestinal contents increases; thus a net diffusive flux into the fish can be maintained. If the fish is unable to excrete this chemical across the gill rapidly enough, the fish can biomagnify its whole-body residues above those that might be expected based on thermodynamic partitioning (also see Connolly and Pedersen 1988).

The simulated dynamics of tetra- and penta-CB bioaccumulation display important qualitative differences depending on the assumed exposure scenario (see Fig. 4 and 5). Although several studies (Norstrom et al. 1976; Niimi 1981; Jensen et al. 1982; Borgmann and Whittle 1983; Spigarelli et al. 1983) have shown that whole-body concentrations of PCBs in fish are generally monotonically increasing functions of the fish's body weight, this pattern of bioaccumulation is predicted only for Scenario II. Even though this trend cannot be discerned in Oliver and Niimi's (1988) Vineland data, it is clearly displayed in their combined Vineland/Credit River data. In particular, using these data the following simple linear regressions were calculated:

$$\log C_f = \begin{cases} 0.823 \log W + 2.67 \ (n = 59;\ r = 0.58) \text{ for tetra-CBs} \\ 1.01 \log W - 0.213 \ (n = 59;\ r = 0.68) \text{ for penta-CBs} \\ 1.11 \log W - 0.649 \ (n = 59;\ r = 0.71) \text{ for hexa-CBs} \\ 1.12 \log W - 0.998 \ (n = 59;\ r = 0.71) \text{ for hepta-CBs.} \end{cases}$$

These regressions suggest that a larger sample of Vineland fish also would show that PCB concentrations are increasing functions of body size. Consequently, Scenario II seems to be more feasible than Scenario I as a realistic representation of PCB bioaccumulation in Lake Ontario salmonids.

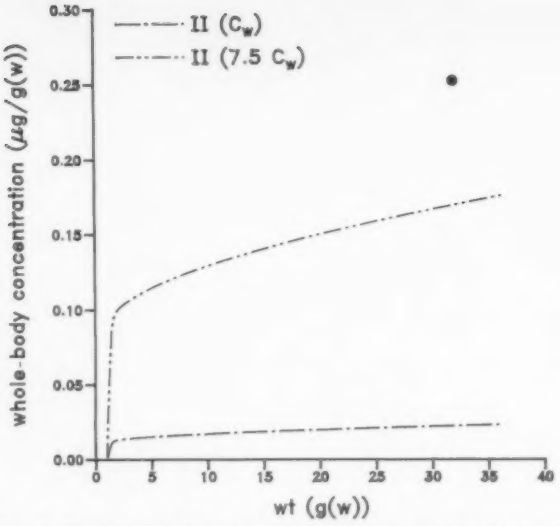


FIG. 8. Simulated whole-body concentration of tetrachlorobiphenyls in Lake Ontario alewife plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

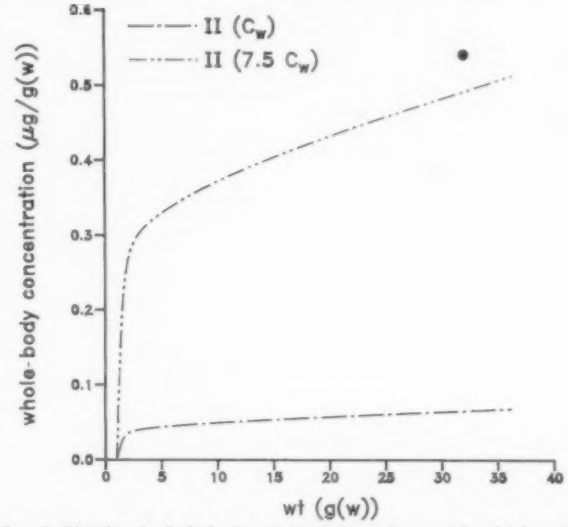


FIG. 9. Simulated whole-body concentration of pentachlorobiphenyls in Lake Ontario alewife plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

Of course the differences in the predicted pattern of tetra- and penta-CB bioaccumulation are the direct result of assuming that whole-body PCB concentrations of alewife are constant rather than dynamic. Figures 8–11 display observed and predicted whole-body concentrations of PCBs in Lake Ontario alewife as a function of their body weight. For both Scenario II (C_w) and Scenario II (7.5 C_w), predicted PCB concentrations in alewife are again increasing functions of the fish's body weight. Assuming that these dynamics are indeed correct, it is difficult to attribute any significance to Scenario I other than

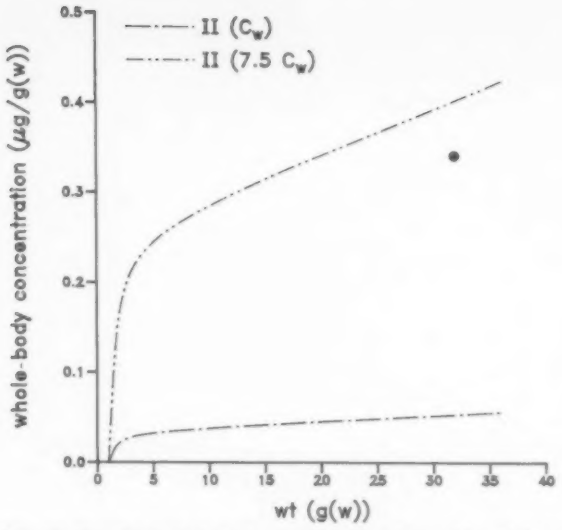


FIG. 10. Simulated whole-body concentration of hexachlorobiphenyls in Lake Ontario alewife plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

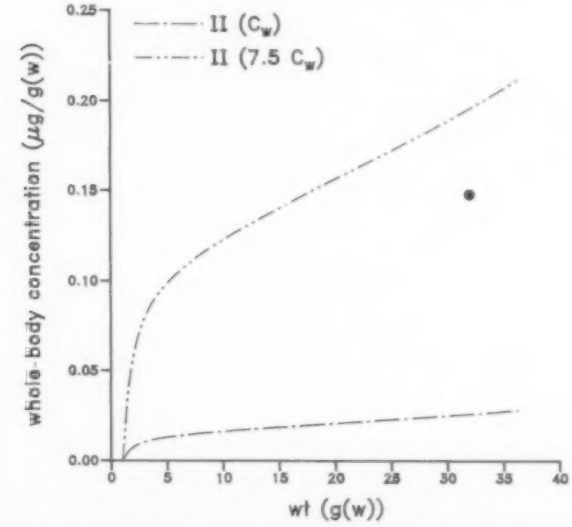


FIG. 11. Simulated whole-body concentration of heptachlorobiphenyls in Lake Ontario alewife plotted against body weight. Observed data are from Oliver and Niimi (1988) and A. J. Niimi (pers. comm.).

as an indication of FGETS's sensitivity to different exposure scenarios. As was the case for the salmonids, whole-body concentrations of PCB in alewife simulated for Scenario II (C_w) are significantly lower than the observed data. Concentrations predicted for Scenario II (7.5 C_w), however, agree reasonably well with the observed data.

The final predicted alewife *BMF* for tetra-, penta-, hexa-, and hepta-CBs are 1.1, 1.2, 1.2, and 1.1, respectively, regardless of assumed aqueous concentrations. According to the preceding explanation for the cause of biomagnification, the prediction that alewifes will biomagnify PCBs less than will salmonids probably is due to their relative gill areas. In particular, the weight-specific gill areas calculated for alewife were

TABLE 8. Summary of mass fluxes predicted by FGETS for a 3-yr simulation of PCB bioaccumulation in Lake Ontario salmonids assuming constant PCB concentration in alewife as reported in Oliver and Niimi (1988). Initial weight of simulated salmonid is 25 g (w). Note that the uptake designated as "accumulated from food" is a net flux, $J_i = C_p F - C_e E$ (i.e. equation 27).

Mass flux	C_w	7.5 C_w
Total ingestion (g)	6677	Same
Total egestion (g)	1695	Same
Total excretion (g)	318	Same
Total respiration (g) (i.e. $R+SDA$)	2217	Same
tetra-CB accumulated by gills (μg)	80.7	605
tetra-CB excreted by gills (μg)	1025	1298
tetra-CB accumulated from food (μg)	1607	1587
tetra-CB egested with feces (μg)	82.3	102
penta-CB accumulated by gills (μg)	97.4	730
penta-CB excreted by gills (μg)	1326	1521
penta-CB accumulated from food (μg)	3323	3284
penta-CB egested with feces (μg)	289	329
hexa-CB accumulated by gills (μg)	34.5	259
hexa-CB excreted by gills (μg)	463	470
hexa-CB accumulated from food (μg)	2031	2012
hexa-CB egested with feces (μg)	248	267
hepta-CB accumulated by gills (μg)	7.47	56.1
hepta-CB excreted by gills (μg)	81.7	85.0
hepta-CB accumulated from food (μg)	859	854
hepta-CB egested with feces (μg)	131	136

approximately 10 times those estimated for salmonids. Thus, alewifes presumably can excrete a greater portion of their accumulated chemical burdens than can salmonids. Because gill areas are generally thought to be correlated with activity levels and trophic position (de Jager and Dekkers 1976; Galis and Barel 1980), this explanation is consistent with the generally accepted belief that differential biomagnification is due to trophic position.

Analysis of Gill versus Food Uptake

There is a continuing debate in the literature regarding the relative significance of gill and food uptake as routes of exposure to fish in polluted ecosystems. Several authors (Weininger 1978; Oliver and Niimi 1983; Spigarelli et al. 1983; Thomann and Connolly 1984) have concluded that gill uptake can account for ony a few percent of the total uptake of hydrophobic chemicals like PCBs and hexachlorobenzene in Great Lakes salmonids. Intuitively, however, the relative significance of one route of uptake to another should depend on the exposure scenario being considered. Indeed, Rhead and Perkins (1984) demonstrated that for goldfish (*Carassius auratus*) bioaccumulating differentially labeled DDT, either gill or food could be the predominant route of uptake depending on prevailing exposure conditions. Because FGETS keeps track of each route of uptake and excretion, analysis of the relative routes of exposure to a fish is very easy and offers another avenue to validate simulations in Figs. 4–11.

For salmonids, FGETS predicts that uptake from food is the predominant route of PCB bioaccumulation. For Scenario I (C_w), gill uptake is predicted to contribute only 5, 3, 2, and 1% of a salmonid's total uptake of tetra-, penta-, hexa-, and hepta-CB, respectively (see Table 8). Although these predictions agree with those of Weininger (1978) and Thomann and Connolly (1984), as aqueous concentrations for Scenario I are increased, the predicted contribution of gill uptake increases

TABLE 9. Summary of salmonid mass fluxes predicted by FGETS for a 3-yr simulation of PCB bioaccumulation in Lake Ontario salmonids assuming dynamic predator prey interaction between salmonids and alewife and dynamic bioaccumulation of PCB in alewife (also see Table 10). Initial weight of simulated salmonid is 25 g (w). Note that the uptake designated as "accumulated from food" is a net flux, $J_i = C_p F - C_e E$ (i.e. equation 27).

Mass flux	C_w	7.5 C_w
Total ingestion (g)	6677	Same
Total egestion (g)	1695	Same
Total excretion (g)	318	Same
Total respiration (g) (i.e. $R+SDA$)	2217	Same
tetra-CB accumulated by gills (μg)	80.7	605
tetra-CB excreted by gills (μg)	120	897
tetra-CB accumulated from food (μg)	130	975
tetra-CB egested with feces (μg)	9.09	68.2
penta-CB accumulated by gills (μg)	97.4	730
penta-CB excreted by gills (μg)	166	1243
penta-CB accumulated from food (μg)	367	2750
penta-CB egested with feces (μg)	34.7	260
hexa-CB accumulated by gills (μg)	34.5	259
hexa-CB excreted by gills (μg)	61.1	458
hexa-CB accumulated from food (μg)	288	2160
hexa-CB egested with feces (μg)	33.4	250
hepta-CB accumulated by gills (μg)	7.47	56.1
hepta-CB excreted by gills (μg)	11.5	86.5
hepta-CB accumulated from food (μg)	134	1005
hepta-CB egested with feces (μg)	17.6	132

significantly. Thus, for Scenario I (7.5 C_w), gill uptake contributes 28, 18, 11, and 6% of a salmonid's total uptake of tetra-, penta-, hexa-, and hepta-CB, respectively (see Table 8). For Scenario II, direct gill exchange contributes 38, 21, 11, and 5% of a salmonid's total uptake of tetra-, penta-, hexa-, and hepta-CB, respectively, regardless of the assumed aqueous concentrations of PCBs (see Table 9). Because we believe Scenario II is ecologically more realistic than Scenario I, these results indicate that although uptake from food is still the major route of exposure, gill exchange of PCBs by Great Lakes salmonids is much more significant than previously thought. One should also realize that this conjecture is independent of the fact that the net flux of PCBs across the gills of these fish is predicted to be negative.

Note also that the uptake designated as being "accumulated from food" in Tables 8 and 9 is the net flux of chemical exchanged across the intestine (i.e. equation 27). In principle, these fluxes included not only chemical that is accumulated directly from food but also chemical that was excreted into the intestine and subsequently reabsorbed. Consequently, because these fuxes include chemical that initially entered the fish via the gills, the preceding comparison of gross gill uptake with net food uptake actually may overestimate the contribution of food. For example, if gross dietary assimilation efficiencies

$$(74)\quad \hat{\alpha}_c = \frac{\int J_i\, dt}{\int C_p I\, dt}$$

are calculated for Scenario I (C_w) using Tables 7 and 8, one would estimate that $\hat{\alpha}_c$ equals 0.95, 0.92, 0.89, and 0.87 for tetra-, penta-, hexa-, and hepta-CB, respectively, and these values are much greater than most of the dietary assimilation efficiencies that have been reported for PCBs in fish (Niimi and Oliver 1987; Gobas et al. 1988; Opperhuizen and Schrap 1988).

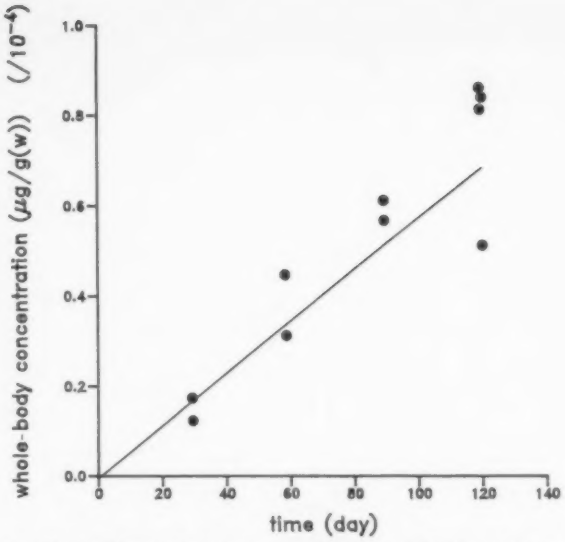


FIG. 12. Simulated whole-body concentration of TCDD in 30-g (w) lake trout. Observed data are from Batterman et al. (1989).

Studies that have quantified the relative contributions of gill and food uptake for hydrophobic chemicals using differentially labeled radioisotopes in laboratory exposures analogous to Scenario II corroborate the conjecture regarding the underestimated significance of direct gill exchange. In paticular, Jarvinen et al. (1977) demonstrated that gill uptake contributes approximately 70% of the total residue of DDT (log $K_{ow} \approx 6.0$) in fathead minnows (*Pimephales promelas*) feeding on clams that are equilibrated with the prevailing aqueous exposure concentration. Similarly, Fisher et al. (1986) showed that direct gill uptake accounts for approximately 60% of a fish's total body burden of kepone (log K_{ow} = 5.3, Ellington and Stancil 1988) when spot (*Leiostomus xanthurus*) feed on grass shrimp (*Paleomontes pugio*) that are likewise equilibrated. Consequently, the relative contribution of gill uptake (i.e. 38%) predicted by FGETS for salmonids bioaccumulating tetra-CBs, which have log K_{ow}s somewhat higher than those of DDT and kepone, seems reasonable.

Batterman et al. (1989) studied the routes of uptake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (log K_{ow} = 7.7) in lake trout using ^{13}C-labeled food and unlabeled sediments. After 120 d, approximately 20% of the fish's total body burden was unlabeled TCDD. Although Batterman et al. discussed the possibility that this accumulation could result from direct gill uptake of dissolved TCDD which is in equilibrium with TCDD in the organic fraction of the sediment, they indicated that the accumulation of unlabeled TCDD was more likely due to direct contact with contaminated sediments via inhalation of suspended sediment or incidental ingestion of bottom sediment during nominal feeding. FGETS simulations of this study, however, suggest that the observed accumulation of unlabeled TCDD, in fact, can be explained by direct gill uptake. In particular, in this study, 30-g (w) lake trout were exposed to a theoretical dissolved concentration of TCDD approximately equal to 0.75 pg/L (see Lodge and Cook 1989) and fed daily a maintenance ration of smelt whose mean TCDD concentration was 77 pg/g (w). We parameterized FGETS to simulate this exposure scenario assuming that lake trout have the same gill morphometry as rainbow trout. Because fish were fed main-

‹enance rations, we also parameterized FGETS to back-calculate the fish's feeding, specific dynamic action, excretion, and egestion assuming a zero specific growth rate and the basal oxygen consumption and assimilation efficiency reported in Table 5. Figure 12 displays the resulting simulation. Not only is the agreement between the simulated bioaccumulation and Batterman and co-workers' observed data remarkable, but also this simulation's prediction that approximately 30% of the lake trout uptake is via direct gill uptake agrees reasonably well with observed 20% accumulation of unlabeled TCDD. Thus, it is reasonable to assume that the uptake of TCDD attributed to direct sediment contact by Batterman et al. was in fact due to direct gill exchange. Because TCDD has a molar volume, and hence an aqueous diffusivity similar to tetra-CBs, but a log K_{ow} more like hepta- or even octa-CBs, the relative importance of gill uptake predicted by FGETS for tetra-CBs (i.e. 38%) and hepta-CBs (i.e. 5%) seems to be consistent with our interpretation of the results of Batterman et al.

Interpretation of results of studies that have attempted to quantify the relative significance of food and water exposures without using isotopes is dependent on the conceptual model one uses to analyze them. Spigarelli et al. (1983), for example, compared whole-body concentrations of PCBs in two groups of brown trout. Both groups were exposed to nominally polluted Lake Michigan water for 57 d. However, whereas one group was fed naturally contaminated alewife, the other group was fed uncontaminated food. Because the whole-body concentrations of the group fed contaminated alewife were nine times higher than that of the fish fed uncontaminated food, Spigarelli et al. concluded that less than 10% of the total uptake of PCBs in brown trout could be attributed to direct aqueous exchange. Such results, however, can be interpreted differently using the model outlined earlier in equations 31, 32, and 33. Because the fish in these two experimental groups presumably fed and grew at the same rate, their apparent rate of elimination, k_2, in equation 33 should have been approximately equal. Hence, the ratio of their whole-body concentrations simply represents the ratio of their respective exposure functions, z, i.e.

$$(75)\quad \frac{C_f(z = k_1 C_w + \phi C_p)}{C_f(z = k_1 C_w)} = 1 + \frac{\phi C_p}{k_1 C_w}.$$

If the final whole-body concentration of fish exposed to both contaminated food and water is nine times that of fish exposed only to contaminated water, equation 75 indicates only that eight times more PCB is ingested than is taken up across the gills. The actual amount of PCB accumulated from food, of course, will be less than this value.

The relative contribution of gill and food uptake obviously will depend greatly on the fish's feeding rate and gill area. Recalling that the weight-specific gill areas of alewife are approximately 10 times those of salmonids, it is not surprising that FGETS predicts that direct gill uptake is the predominant route of PCB bioaccumulation for alewife. In particular, assuming that alewife feed on zooplankton/*Mysis* that are equilibrated with prevailing water concentrations, FGETS predicts that direct gill exchange accounts for 87, 80, 72, and 62% of an alewife's total uptake of tetra-, penta-, hexa-, and hepta-CB, respectively (see Table 10).

TABLE 10. Summary of mass fluxes for alewife predicted by FGETS for a 3-yr simulation of PCB bioaccumulation in Lake Ontario salmonids assuming dynamic predator prey interaction between salmonids (also see Table 9). Initial weight of simulated alewife is 1 g (w). Note that the uptake designated as "accumulated from food" is a net flux, $J_i = C_p F - C_e E$ (i.e. equation 27).

Mass flux	C_w	7.5 C_w
Total ingestion (g)	631	Same
Total egestion (g)	101	Same
Total excretion (g)	89.5	Same
Total respiration (g) (i.e. $R + SDA$)	405	Same
tetra-CB accumulated by gills (μg)	16.0	120
tetra-CB excreted by gills (μg)	17.6	132
tetra-CB accumulated from food (μg)	2.36	17.7
tetra-CB egested with feces (μg)	0.363	2.72
penta-CB accumulated by gills (μg)	19.4	145
penta-CB excreted by gills (μg)	21.6	162
penta-CB accumulated from food (μg)	4.73	35.5
penta-CB egested with feces (μg)	1.25	9.35
hexa-CB accumulated by gills (μg)	6.86	51.4
hexa-CB excreted by gills (μg)	7.41	55.6
hexa-CB accumulated from food (μg)	2.61	19.6
hexa-CB egested with feces (μg)	1.14	8.52
hepta-CB accumulated by gills (μg)	1.48	11.1
hepta-CB excreted by gills (μg)	1.36	10.2
hepta-CB accumulated from food (μg)	0.898	6.75
hepta-CB egested with feces (μg)	0.592	4.45

Discussion

Although our goal has not been to dispute the standing toxicokinetic paradigm that food chain exposure is often a fish's predominant route of exposure to the more hydrophobic environmental contaminants, our simulations of PCB bioaccumulation in Lake Ontario salmonids suggest that gill uptake probably is more significant than previous studies have indicated. This assertion may be attributed to (1) the gill exchange model used in FGETS, (2) FGETS's fecal partitioning model which predicts that chemical assimilation efficiencies from food decrease as fish bioaccumulate chemicals, or (3) a combination of these factors. Even if the uptake of very hydrophobic chemicals by the gill was insignificant, realistic bioaccumulation models still must represent chemical exchange across gills as accurately as possible because of the gill's ability to excrete such chemicals. For example, under Scenario II, salmonids are predicted to excrete across their gills 57, 36, 19, and 8% of their total uptake of tetra-, penta-, hexa-, and hepta-CB, respectively (see Table 9). Additionally, equation 34 clearly illustrates that if one assumes that chemical exchange across both the gill and the intestine is by diffusion, then dietary uptake assimilation efficiencies are determined in part by the fish's ability to exchange chemicals across the gills.

Our simulations also demonstrate that in order to predict realistic bioaccumulation dynamics under field conditions, one must scrutinize assumed exposure conditions and data very carefully. This is particularly true when attempting to model the bioaccumulation of hydrophobic chemicals because accurate determination of aqueous concentrations of hydrophobic chemicals is difficult regardless of one's expertise and diligence. In fact, this situation has caused some researchers to suggest that water quality and chemical pollution of aquatic ecosystems should be monitored by chemical concentrations in biota and/or sediments rather than by aqueous concentrations per se.

The ability to predict the bioaccumulation of chemicals in fish is of more than passing scientific interest. Although the extensive use of exposure-referenced, toxicological benchmarks such as LC_{50}s and EC_{50}s has obscured the fact that most

adverse ecological effects caused by chemical pollution actually are due to the internal accumulation of pollutants rather than the concentrations of polutants in the environment per se, several authors (Neely 1984; Friant and Henry 1985; McCarty et al. 1985; McCarty 1986) have discussed the need to consider chemical bioaccumulation explicitly when assessing ecological effects of organic pollutants in aquatic and marine ecosystems. Indeed, recent residue-based toxicity studies substantiate this contention. Kobayashi and Kishino (1980), for example, showed that goldfish die due to acute aqueous exposure to pentachlorophenol (PCP) when the fish accumulate a whole-body concentration approximately equal to 0.38 μmol/g (w) regardless of the exposure pH or the PCP concentration. Similarly, van Hoogen and Opperhuizen (1988) demonstrated that actue aqueous exposures of chlorinated benzenes kill guppies (*Poecilia reticulata*) when the fish accumulate a whole-body concentration of approximately 2.5 μmol/g (w) regardless of the congener's degree of chlorination or of the exposure concentration. Opperhuizen and Schrap (1988) showed that chronic dietary exposures to PCBs kill guppies when the fish accumulate a whole-body concentration approximately equal to 0.7 μmol/g (w) regardless of the PCB concentration in the food. Analogous results also have been demonstrated for important sublethal endpoints. For example, Donkin et al. (1989) showed that filtering/feeding rates are reduced to one half their preexposure levels when mussels (*Mytilus edulis*) accumulate a whole-body concentration of 0.160 μmol/g (w) for variously substituted benzenes and polynuclear aromatics.

Even when exposure-referenced, toxicological benchmarks are used in making ecological risk assessments, bioaccumulation models such as FGETS can help to interpret effects data more accurately. Toxicokinetic limitations, for example, can explain the apparent decrease in narcotic toxicity for extremely hydrophobic chemicals in acute toxicity tests (e.g. Veith et al. 1983). Similarly, exchange kinetics explain the apparent increased tolerance of large individuals compared with small individuals irrespective of any intrinsic differential metabolic tolerance (e.g. Kumaraguru and Beamish 1981). If interspecies regressions for LC_{50} relationships are used to make ecological risk assessments, one should ascertain whether observed differences or similarities are due to toxicokinetics or to intrinsic physiological sensitivities. For example, Kulshrestha et al. (1986) studied the relative toxicity of four pesticides on three carp species, *Labeo rohita, Catla catla*, and *Cirrhinus mrigala*, of similar size and showed that for each pesticide tested, the LC_{50}s for *Catla catla* were greater than the LC_{50}s for *Labeo rohita*, which were greater than LC_{50}s for *Cirrhinus mrigala*. Because it seems resonable to expect that closely related species have similar toxicological sensitivities, one might attribute this particular pattern of interspecies sensitivity to differences in toxicokinetics and indeed one would expect these results based on the relative gill areas of these fish (Roy and Munshi 1984; Hughes and Ojha 1986; Pandey et al. 1987).

Acknowledgments

We wish to thank Drs. Arthur Niimi and Barry Oliver for graciously providing us with their residue data for their Lake Ontario ecosystem study. We also thank Drs. Russell Erickson and James McKim of EPA's Environmental Research Laboratory, Duluth, MN, for many constructive discussions during the development of the FGETS model. Finally, we extend our appreciation to Drs. Uwe Borgmann and Geoffrey Evans whose reviews and suggestions added greatly to this paper.

This study was conducted as part of the Ecological Risk Assessment Research Program, a multilaboratory research program of the United States Environmental Protection Agency, Office of Research and Development.

References

Barber, M. C., L. A. Suárez, and R. R. Lassiter. 1988. Modeling bioconcentration of nonpolar organic pollutants by fish. Environ. Toxicol. Chem. 7: 545–558.

Batterman, A. R., P. M. Cook, K. B. Lodge, D. B. Lothenbach, and B. C. Butterworth. 1989. Methodology used for a laboratory determination of the relative contributions of water, sediment, and food chain routes of uptake for 2,3,7,8-TCDD bioaccumulation by lake trout in Lake Ontario. Chemosphere 19: 451–458.

Beamish, F. W. H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature of several species. Can. J. Zool. 42: 177–188.

Beamish, F. W. H., and M. Legrow. 1983. Bioenergetics of the southern brook lamprey, *Ichthyomyzon gagei*. J. Anim. Ecol. 52: 575–590.

Beamish, F. W. H., and P. D. MacMahon. 1988. Apparent heat increment and feeding strategy in walleye, *Stizostedion vitreum vitreum*. Aquaculture 68: 73–82.

Black, M. C., and J. F. McCarthy. 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow trout (*Salmo gairdneri*). Environ. Toxicol. Chem. 7: 593–600.

Boisclair, D., and W. C. Leggett. 1988. An in situ experimental evaluation of the Elliott–Persson and Eggers models for estimating fish daily ration. Can. J. Fish. Aquat. Sci. 45: 138–145.

Booth, J. H. 1978. The distribution of blood flow in the gills of fish: application of a new technique to rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 73: 119–129.

Borgmann, U., and D. M. Whittle. 1983. Particle-size-conversion efficiency and contaminant concentrations in Lake Ontario biota. Can. J. Fish. Aquat. Sci. 40: 328–336.

Brandt, S. B. 1986. Food of trout and salmon in Lake Ontario. J. Great Lakes Res. 12: 200–205.

Branson, D. R., I. T. Takahashi, W. M. Parker, and G. E. Blau. 1985. Bioconcentration kinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rainbow trout. Environ. Toxicol. Chem. 4: 779–788.

Brett, J. R. 1971. Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 409–415.

Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Board Can. 26: 2363–2394.

Brett, J. R., and C. A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 32: 2479–2486.

Briggs, G. G. 1981. Theoretical and experimental relationships between soil adsorption, octanol–water partition coefficients, water solubilities, bioconcentration factors, and the parachor. J. Agric. Food Chem. 29: 1050–1059.

Brown, G. M. 1960. Heat or mass transfer in a fluid in laminar flow in a circular or flat conduit. AIChE J. 6: 179–183.

Brown, Jr., E. H. 1972. Population biology of alewives, *Alosa pseudoharengus*, in Lake Michigan, 1949–1970. J. Fish. Res. Board Can. 29: 477–500.

Byczkowska-Smyk, W. 1957. The respiratory surface of the gills in teleosts. Part I: the respiratory surface of the gills in the flounder, *Pleuronectes platessa*, and perch, *Perca fluviatilis*. Zool. Pol. 7: 90–111.

1958. The respiratory surface of the gills in teleosts. Part II: the respiratory surface of the gills in the eel (*Anguilla anguilla*), the loach (*Misgurnue fossilis* L.), and the perch-pike (*Lucioperca lucioperca*). Acta Biol. Cracov. Ser. Zool. 1: 83–97.

1959a. The respiratory surface of the gills in teleosts. Part III: the respiratory surface of the gills in the tench (*Tinca tinca*), the silver bream (*Blicca bjoerkna*), and the chondrostoma (*Chondrostoma nasus* L.). Acta Biol. Cracov. Ser. Zool. 2: 73–88.

1959b. The respiratory surface of the gills in teleosts. Part IV: the respiratory surface of the gills in the pike (*Esox lucius* L.), stone-perch (*Acerina cerniua* L.), and the burbot (*Lota lota* L.). Acta Biol. Cracov. Ser. Zool. 2: 113–127.

1961. Development of the respiratory surface in the gills of the rainbow-trout (*Salmo irideus* Gibb.). Acta Biol. Cracov. Ser. Zool. 4: 89–109.

CARLANDER, K. 1969. Handbook of freshwater fishery biology. Vol. I. Iowa State University Press, Ames, IA. 752 p.

CHIOU, C. T. 1985. Partition coefficients of organic compounds in lipid–water systems and correlations with fish bioconcentration factors. Environ. Sci. Technol. 19: 57–62.

CHIOU, C. T., R. L. MALCOLM, T. I. BRINTON, AND D. E. KILE. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environ. Sci. Technol. 20: 502–508.

COLTON, C. K., K. A. SMITH, P. STROEVE, AND E. W. MERRILL 1971. Laminar flow mass transfer in a flat duct with permeable walls. AIChE J. 17: 773–780.

CONNELL, D. W. 1989. Biomagnification by aquatic organisms — A proposal. Chemosphere 19: 1573–1584.

CONNOLLY, J. P., AND C. J. PEDERSEN. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environ. Sci. Technol. 22: 99–103.

CRAIG, J. F. 1977. The body composition of the adult perch, *Perca fluviatilis* in Windermere, with reference to seasonal changes and reproduction. J. Anim. Ecol. 46: 617–632.

CZOLOWSKA, R. 1965. The respiratory surface of the gills of the *Alosa kessleri pontica* (Eichwald). Acta Biol. Cracov. Ser. Zool. 8: 101–117.

DAVIS, J. C., AND D. J. RANDALL. 1973. Gill irrigation and pressure relationships in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 99–104.

DE JAGER, S., AND W. J. DEKKERS. 1976. Relations between gill structure and fish activity. Neth. J. Zool. 25: 276–308.

DE LA NOUE, J., AND G. CHOUBERT. 1986. Digestibility in rainbow trout: comparison of direct and indirect methods of measurement. Prog. Fish-Cult. 48: 190–195.

DONKIN, P., J. WIDDOWS, S. V. EVANS, C. M. WORRALL, AND M. CARR. 1989. Quantitative structure–activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). Aquat. Toxicol. 14: 277–294.

DRENNER, R. W., W. J. O'BRIEN, AND J. R. MUMMERT. 1982. Filter-feeding rates of gizzard shad. Trans. Am. Fish. Soc. 111: 210–215.

DUBE, S. C., AND J. S. D. MUNSHI. 1974. Studies on the blood–water diffusion barrier of secondary gill lamellae of an air-breathing fish, *Anabas testudineus* (Bloch). Zool. Anz. Jena 193: 35–41.

DUNBRACK, R. L. 1988. Feeding of juvenile coho salmon (*Oncorhynchus kisutch*): maximum appetite, sustained feeding rate, appetite return, and body size. Can. J. Fish. Aquat. Sci. 45: 1191–1196.

ELLINGTON, J. J., AND F. E. STANCIL, JR. 1988. Octanol/water partition coefficients for evaluation of hazardous waste land disposal: Selected chemicals. EPA/600/M-88/010. U.S.A. Environmental Protection Agency, Athens, GA.

ELLIOTT, J. M. 1975. Weight of food and time required to satiate brown trout, *Salmo trutta* L. Freshwater Biol. 5: 51–64.

1976. Body composition of the brown trout (*Salmo trutta* L.) in relation to temperature and ration size. J. Anim. Ecol. 45: 273–289.

ELLIOTT, J. M., AND L. PERSSON. 1978. The estimation of daily rates of food consumption for fish. J. Anim. Ecol. 47: 977–991.

ESCHMEYER, P. H., AND A. M. PHILLIPS. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. Trans. Am. Fish. Soc. 94: 62–74.

FISHER, D. J., J. R. CLARK, M. H. ROBERTS, JR., J. P. CONNOLLY, AND L. H. MUELLER. 1986. Bioaccumulation of kepone by spot (*Leiostomus xanthurus*): importance of dietary accumulation and ingestion rate. Aquat. Toxicol. 9: 161–178.

FLATH, L. E., AND J. S. DIANA. 1985. Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Trans. Am. Fish. Soc. 114: 328–337.

FLINT, R. W. 1986. Hypothesized carbon flow through the deepwater Lake Ontario food web. J. Great Lakes Res. 12: 344–354.

FRIANT, S. L., AND L. HENRY. 1985. Relationship between toxicity of certain organic compounds and their concentrations in tissues of aquatic organisms: a perspective. Chemosphere 14: 1897–1907.

GALIS, F., AND C. D. N. BAREL. 1980. Comparative functional morphology of the gills of African lacustrine Cichlidae (Pisces, Teleostei). Neth. J. Zool. 30: 392–430.

GALLAGHER, M. L., E. KANE, AND J. COURTNEY. 1984. Differences in oxygen consumption and ammonia production among American elvers (*Anguilla rostrata*). Aquaculture 40: 183–187.

GARBER, K. J. 1983. Effect of fish size, meal size and dietary moisture on gastric evacuation of pelleted diets by yellow perch, *Perca flavescens*. Aquaculture 34: 41–49.

GEHRKE, P. C. 1987. Cardio-respiratory morphometrics of the spangled perch *Leiopotherapon unicolor* (Gunter, 1859) (Percoidei, Teraponidae). J. Fish. Biol. 31: 617–623.

GOBAS, F. A. P. C., AND D. MACKAY. 1987. Dynamics of hydrophobic organic chemical bioconcentration in fish. Environ. Toxicol. Chem. 6: 495–504.

GOBAS, F. A. P. C., D. C. G. MUIR, AND D. MACKAY. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17: 943–962.

GOBAS, F. A. P. C., A. OPPERHUIZEN, AND O. HUTZINGER. 1986. Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation. Environ. Toxicol. Chem. 5: 637–646.

GRABNER, M., AND R. HOFER. 1985. The digestibility of the proteins of broad bean (*Vicia faba*) and soya bean (*Glycine max*) under in vitro conditions simulating the alimentary tracts of rainbow trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*). Aquaculture 48: 111–122.

GRIMSRUD, L., AND A. L. BABB. 1966. Velocity and concentration profiles for laminar flow of a Newtonian fluid in a dialyzer. Chem. Eng. Progr. Symp. Ser. No. 66, 62: 19–31.

GROVE, D. J., L. G. LOIZIDES, AND J. NOTT. 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. J. Fish Biol. 12: 507–516.

GROVES, T. D. D. 1970. Body composition changes during growth in young sockeye (*Oncorhynchus nerka*) in fresh water. J. Fish. Res. Board Can. 27: 929–942.

GRUGER, JR., E. H., N. L. KARRICK, A. I. DAVIDSON, AND T. HRUBY. 1975. Accumulation of 3,4,3′,4′-tetrachlorobiphenyl and 2,4,5,2′,4′,5′- hexachlorobiphenyl in juvenile coho salmon. Environ. Sci. Technol. 9: 121–127.

GRZENDA, A. R., D. F. PARIS, AND W. J. TAYLOR. 1970. The uptake, metabolism, and elimination of chlorinated residues by goldfish (*Carassius auratus*) fed a 14C-DDT contaminated diet. Trans. Am. Fish. Soc. 99: 385–396.

HAWKER, D. W., AND D. W. CONNELL. 1988. Octanol–water partition coefficients of polychlorinated biphenyl congeners. Environ. Sci. Technol. 22: 382–387.

HAYDUK, W., AND H. LAUDIE. 1974. Prediction of diffusion coefficients for nonelectrolytes in dilute aqueous solutions. AIChE J. 20: 611–615.

HILLS, B. A., AND G. M. HUGHES. 1970. A dimensional analysis of oxygen transfer in fish gills. Respir. Physiol. 9: 126–140.

HOLLING, C. S. 1966. The functional response of invertebrate predators to prey density. Mem. Entomol. Soc. Can. 48: 1–86.

HUGHES, G. M. 1966. The dimensions of fish gills in relation to their function. J. Exp. Biol. 45: 177–195.

1972. Morphometrics of fish gills. Respir. Physiol. 14: 1–25.

1984. General anatomy of the gills. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol XA. Academic Press, New York, NY. 456 p.

HUGHES, G. M., AND M. MORGAN. 1973. The structure of fish gills in relation to their respiratory function. Biol. Rev. 48: 419–475.

HUGHES, G. M., AND J. OJHA. 1986. Critical study of gill area of a free-swimming river carp, *Catla catla*, and an air-breathing perch, *Anabas testudineus*. Proc. Indian Natl. Sci. Acad. B51: 391–404.

HUGHES, G. M., S. F. PERRY, AND J. PIIPER. 1986. Morphometry of the gills of the elasmobranch *Scyliorhinus stellaris* in relation to body size. J. Exp. Biol. 121: 27–42.

JAFFE, R., AND R. A. HITES. 1986. Fate of hazardous waste derived organic compounds in Lake Ontario. Environ. Sci. Technol. 20: 267–274.

JAKUBOWSKI, M. 1977. Size and vascularization of the respiratory surface of the gills and skin in some cobitids (Cobitidae, Pisces). Bull. Acad. Pol. Sci. Ser. Sci. Biol. 25: 307–316.

1982. Size and vascularization of the gill and skin respiratory surfaces in the white amur, *Ctenopharyngodon idella* (Val.) (Pisces, Cyprinidae). Acta Biol. Cracov. Ser. Zool. 24: 93–106.

JARVINEN, A. W., M. J. HOFFMAN, AND T. W. THORSLUND. 1977. Long-term toxic effects of DDT food and water exposure on fathead minnows (*Pimephales promelas*). J. Fish. Res. Board Can. 34: 2089–2103.

JENSEN, A. L. 1986. Functional regression and correlation analysis. Can. J. Fish. Aquat. Sci. 43: 1742–1745.

JENSEN, A. L., S. A. SPIGARELLI, AND M. M. THOMMES. 1982. PCB uptake by five species of fish in Lake Michigan, Green Bay of Lake Michigan, and Cayuga Lake, New York. Can. J. Fish. Aquat. Sci. 39: 700–709.

JOBLING, M. 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. J. Fish Biol. 19: 245–257.

1987. Influence of particle size and dietary energy content on patterns of gastric evacuation in fishes: test of a physiological model of gastric emptying. J. Fish Biol. 30: 299–314.

JUDE, D. J., F. J. TESAR, S. F. DEBOE, AND T. J. MILLER. 1987. Diet and selection of major prey species by Lake Michigan salmonines, 1973–1982. Trans. Am. Fish. Soc. 116: 677–691.

KARICKHOFF, S. W. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soil. Chemosphere 10: 833–846.

KLEEMAN, J. M., J. R. OLSON, S. M. CHEN AND R. E. PETERSON. 1986a. Metabolism and deposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rainbow trout. Toxicol. Appl. Pharmacol. 83: 391–401.

1986b. Metabolism and deposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in yellow perch. Toxicol. Appl. Pharmacol. 83: 402–411.

KOBAYASHI, K., AND T. KISHINO. 1980. Effect of pH on the toxicity and accumulation of pentachlorophenol in goldfish. Bull. Jpn. Soc. Sci. Fish. 46: 167–170.

KULSHRESTHA, S. K., N. ARORA, AND S. SHARMA. 1986. Toxicity of four pesticides on the fingerlings of Indian major carps, *Labeo rohita, Catla catla*, and *Cirrhinus mrigala*. Ecotoxicol. Environ. Saf. 12: 114–119.

KUMARAGURU, A. K., AND F. W. H. BEAMISH. 1981. Lethal toxicity of permethrin (NRDC-143) to rainbow trout, *Salmo gairdneri*, in relation to body weight and water temperature. Water Res. 15: 503–505.

KUNISAKI, N., K. TAKADA, AND H. MATSUURA. 1986. On the study of lipid content, muscle hardness, and fatty acid composition of wild and cultured horse mackerel. Bull. Jpn. Soc. Sci. Fish. 52: 333–336.

KUTTY, M. N. 1968. Respiratory quotients in goldfish and rainbow trout. J. Fish. Res. Board Can. 25: 1689–1728.

LANDOLT, J. C., AND L. G. HILL. 1975. Observations of the gross structures and dimensions of the gills of three species of gars (Lepisoteidae). Copeia 1975(3): 470–475.

LEWIS, S. V., AND I. C. POTTER. 1976. Gill morphometrics of the lampreys *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). Acta Zool. (Stockh.) 57: 103–112.

LIEB, A. J., D. D. BILLS, AND R. O. SINNHUBER. 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout (*Salmo gairdneri*). J. Agric. Food Chem. 22: 638–642.

LISZKA, E. 1969. The vascularization and respiratory surface of the gills in the river bullhead (*Cottus gobio*). Acta Biol. Cracov. Ser. Zool. 12: 135–148.

LODGE, K. B., AND P. M. COOK. 1989. Partitioning studies of dioxin between sediment and water: The measurement of K_{oc} for Lake Ontario sediment. Chemosphere 19: 439–444.

LORETZ, C. A. 1979. Water exchange across fish gills: the significance of tritiated water flux measurements. J. Exp. Biol. 79: 147–162.

LOWE, T. P., T. W. MAY, W. G. BRUMBAUGH, AND D. A. KANE. 1985. National Contaminant Biomonitoring Program: concentrations of seven elements in freshwater fish, 1979–1981. Arch. Environ. Contam. Toxicol. 14: 363–388.

MACHIELS, M. A. M., AND A. M. HENKEN. 1986. A dynamic simulation model for the growth of the African catfish, *Clarias gariepinus* (Burchell 1822). I. Effect of feeding level on growth and energy metabolism. Aquaculture 56: 29–52.

1987. A dynamic simulation model for the growth of the African catfish, *Clarias gariepinus* (Burchell 1822). II. Effect of feed composition on growth and energy metabolism. Aquaculture 60: 33–53.

MACKAY, D. 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16: 274–278.

1989. Modeling the long-term behavior of an organic contaminant in a large lake: application to PCBs in Lake Ontario. J. Great Lakes Res. 15: 283–297.

MACKAY, D., AND A. I. HUGHES. 1984. Three-parameter equation describing the uptake of organic compounds by fish. Environ. Sci. Technol. 18: 430–444.

MARAIS, J. F. K., AND T. ERASMUS. 1977. Chemical composition of the alimentary canal contents of mullet (Teleostei:Mugilidae) caught in the Swartkops Estuary near Port Elizabeth, South Africa. Aquaculture 10: 263–273.

MCCARTY, L. S. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ. Toxicol. Chem. 5: 1071–1080.

MCCARTY, L. S., P. V. HODSON, G. C. CRAIG, AND K. L. E. KAISER. 1985. The use of quantitative structure–activity relationships to predict the acute and chronic toxicities of organic chemicals to fish. Environ. Toxicol. Chem. 4: 595–606.

MORISHITA, T., U. KAZUAKI, N. IMURA, AND T. TAKAHASHI. 1987. Variation with growth in the proximate compositions of cultured red sea bream. Bull. Jpn. Soc. Sci. Fish. 53: 1601–1607.

MUIR, B. S., AND C. E. BROWN. 1971. Effects of blood pathway on the blood-pressure drop in fish gills, with special reference to tunas. J. Fish. Res. Board Can. 28: 947–955.

MULLER-FEUGA, A., J. PETIT, AND J. J. SABAUT. 1978. The influence of temperature and wet weight on the oxygen demand of rainbow trout (*Salmo gairdneri* R.) in fresh water. Aquaculture 14: 355–363.

MURPHY, P. G., AND J. V. MURPHY. 1971. Correlations between respiration and direct uptake of DDT in the mosquito fish *Gambusia affinis*. Bull. Environ. Contam. Toxicol. 6: 581–588.

NEELY, W. B. 1984. An analysis of aquatic toxicity data: water solubility and acute LC_{50} fish data. Chemosphere 13: 813–819.

NIIMI, A. J. 1981. Gross growth efficiency of fish (K_1) based on field observations of annual growth and kinetics of persistent environmental contaminants. Can. J. Fish. Aquat. Sci. 38: 250–253.

NIIMI, A. J., AND S. L. MORGAN. 1980. Morphometric examination of the gills of walleye, *Stizostedion vitreum vitreum* (Mitchill) and rainbow trout, *Salmo gairdneri* (Richardson). J. Fish Biol. 16: 685–692.

NIIMI, A. J., AND B. G. OLIVER. 1987. Influence of molecular weight and molecular volume on the dietary absorption efficiency of chemicals by fish. Can. J. Fish. Aquat. Sci. 45: 222–227.

NORHEIM, G., AND S. O. ROALD. 1985. Distribution and elimination of hexachlorobenzene, octachlorostyrene, and decachlorobiphenyl in rainbow trout, *Salmo gairdneri*. Aquat. Toxicol. 6: 13–24.

NORSTROM, R. J., A. E. MCKINNON, AND A. S. W. DEFREITAS. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). J. Fish. Res. Board Can. 33: 248–267.

OIKAWA, S., AND Y. ITAZAWA. 1985. Gill and body surface areas of the carp in relation to body mass, with special reference to the metabolic-size relationship. J. Exp. Biol. 117: 1–14.

OLIVER, B. G., AND A. J. NIIMI. 1983. Bioconcentration of chlorobenzene from water by rainbow trout: correlations with partition coefficients and environmental residues. Environ. Sci. Technol. 17: 267–291.

1985. Bioconcentration factors of some halogenated organics for rainbow trout: limitations in their use for prediction of environmental residues. Environ. Sci. Technol. 19: 842–849.

1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ. Sci. Technol. 22: 388–937.

OLSON, R. J., AND A. J. MULLEN. 1986. Recent developments for making gastric evacuation and daily ration determinations. Environ. Biol. Fishes 16: 183–191.

OPPERHUIZEN, A., AND S. M. SCHRAP. 1988. Uptake efficiencies of two polychlorobiphenyls in fish after dietary exposure to five different concentrations. Chemosphere 17: 253–262.

PALING, J. E. 1968. A method of estimating the relative volumes of water flowing over the different gills of a freshwater fish. J. Exp. Biol. 48: 533–544.

PALOHEIMO, J. E., and L. M. DICKIE. 1965. Food and growth of fishes. I. A growth curve derived from experiment data. J. Fish. Res. Board Can. 22: 521–542.

PANDEY, A., G. K. KUNWAR, and J. S. D. MUNSHI. 1987. Comparative study of the gill surface area of the Indian Shad *Hilsa ilisha* (Ham.) and a major carp *Labeo rohita* (Ham.). Proc. Indian Natl. Sci. Acad. B53: 205–214.

PATTON, J. S., B. STONE, C. PAPA, R. ABRAMOWITZ, and S. H. YALKOWSKY. 1984. Solubility of fatty acids and other hydrophobic molecules in liquid trioleoylglycerol. J. Lipid Res. 25: 189–197.

PAULSON, L. J. 1980. Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1421–1425.

PIIPER, J., P. SCHEID, S. F. PERRY, AND G. M. HUGHES. 1986. Effective and morphological oxygen-diffusing capacity of the gills of the elasmobranch *Scyliorhinus stellaris*. J. Exp. Biol. 123: 27–41.

PRICE, J. W. 1931. Growth and gill development in the small mouthed black bass, *Micropterus dolomieui* Lacepede. Stud. Ohio State Univ. 4: 46 p.

RASHEVSKY, N. 1959. Some remarks on the mathematical theory of nutrition of fishes. Bull. Math. Biophys. 21: 161–183.

RAYNER, J. M. V. 1985. Linear relations in biomechanics: the statistics of scaling functions. J. Zool. Lond. (A) 206: 415–439.

RHEAD, M. M., and J. M. PERKINS. 1984. An evaluation of the relative importance of food and water as sources of p,p' – DDT to the goldfish, *Carassius auratus* (L.). Water Res. 18: 719–725.

ROY, P. K., and J. S. D. MUNSHI. 1984. Oxygen uptake in relation to body weight and respiratory surface area in *Cirrhinus mrigala* at two different seasonal temperatures. Proc. Indian Natl. Sci. Acad. B50: 387–394.

RUGGERONE, G. T. 1989. Gastric evacuation rates and daily ration of piscivorous coho salmon, *Oncorhynchus kisutch* Walbaum. J. Fish Biol. 34: 451–463.

SAUNDERS, R. L. 1962. The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow activity and concentrations of oxygen and carbon dioxide. Can. J. Zool. 40: 817–862.

SCAVIA, D. 1980. An Ecological model of Lake Ontario. Ecol. Model. 8: 49–78.

SCHEID, P., AND J. PIIPER. 1971. Theoretical analysis of respiratory gas equilibrium in water passing through fish gills. Respir. Physiol. 13: 305–318.

SERVOS, M. R., D. C. G. MUIR, and G. R. B. WEBSTER. 1989. Effect of dissolved organic matter on bioavailability of polychlorinated dibenzo-*p*-dioxins. Aquat. Toxicol. 14: 169–184.

SHUBINA, L. I., and T. L. RYCHAGOVA. 1981. Dynamics of fat and water metabolism in Caspian shad, *Alosa caspia caspia*, in relation to its biological peculiarities. J. Ichthyol. 21(6): 123–127.

SIDELL, B. D., and J. R. HAZEL. 1987. Temperature affects the diffusion of small molecules the cytosol of fish muscle. J. Exp. Biol. 129: 191–203.

SIMONS, T. J., C. R. MURTHY, and J. E. CAMPBELL. 1985. Winter circulation in Lake Ontario. J. Great Lakes Res. 11: 423–433.

SPIGARELLI, S. A., M. M. THOMMES, and W. PREPEJCHAL. 1983. Thermal and metabolic factors affecting PCB uptake by adult brown trout. Environ. Sci. Technol. 17: 88–94.

STAPLES, D. J., and M. NOMURA. 1976. Influence of body size and food ration on the energy budget of rainbow trout *Salmo gairdneri* Richardson. J. Fish Biol. 9: 29–43.

STARMACH, J. 1971. Oxygen consumption and respiratory surface of gills in *Cottus poecilopus* Heckel and *Cottus gobio* L. Acta Biol. Cracov. Ser. Zool. 14: 9–15.

STEEN, J. B., and T. BERG. 1966. The gills of two species of haemoglobin-free fishes compared to those of other teleosts — with a note on severe anaemia in an eel. Comp. Biochem. Physiol. 18: 517–526.

STEVENS, E. D., and E. N. LIGHTFOOT. 1986. Hydrodynamics of water flow in front of and through the gills of skipjack tuna. Comp. Biochem. Physiol. 83A: 255–259.

STEWART, D. J., and F. P. BINKOWSKI. 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: an energetics-modeling synthesis. Trans. Am. Fish. Soc. 115: 643–659.

STEWART, D. J., D. WEININGER, D. V. ROTTIERS, and T. A. EDSALL. 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. Can. J. Fish. Aquat. Sci. 40: 681–698.

TANDLER, A., and F. W. H. BEAMISH. 1981. Apparent specific dynamic action (SDA), fish weight and level of caloric intake in the largemouth bass, *Micropterus salmoides*. Aquaculture 23: 231–242.

THOMANN, R. V. 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38: 280–296.

1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23: 699–707.

THOMANN, R. V., and J. P. CONNOLLY. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18: 65–71.

UMEZAWA, S., and H. WATANABE. 1973. On the respiration of the killifish *Oryzias latipes*. J. Exp. Biol. 58: 305–325.

URSIN, E. 1967. A mathematical model of some aspects of fish growth, respiration, and mortality. J. Fish. Res. Board Can. 24: 2355–2390.

VAN HOOGEN, G., and A. OPPERHUIZEN. 1988. Toxicokinetics of chlorobenzenes in fish. Environ. Toxicol. Chem. 7: 213–219.

VAN VELD, P. A., M. E. BENDER, and M. H. ROBERTS, JR. 1984. Uptake, distribution, and clearance of chlordecone by channel catfish (*Ictalurus punctatus*). Aquat. Toxicol. 5: 33–49.

VEITH, G. D., D. J. CALL, and L. T. BROOKE. 1983. Structure–toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. 40: 743–748.

VETTER, R. D., M. C. CAREY, and J. S. PATTON. 1985. Coassimilation of dietary fat and benzo(*a*)pyrene in the small intestine: an absorption model using the killifish. J. Lipid Res. 26: 428–434.

WALKER, G., AND T. DAVIES. 1974. Mass transfer in laminar flow between parallel permeable plates. AIChE J. 20: 881–889.

WARE, D. M. 1975. Growth, metabolism, and optimal swimming speed of a pelagic fish. J. Fish. Res. Board Can. 32: 33–41.

WEATHERLEY, A. H., and H. S. GILL. 1983. Protein, lipid, water, and caloric contents of immature rainbow trout, *Salmo gairdneri* Richardson, growing at different rates. J. Fish Biol. 23: 653–673.

WEININGER, D. 1978. Accumulation of PCBs by lake trout in Lake Michigan. Ph.D. thesis, University of Wisconsin-Madison, Madison, WI. 232 p.

WRIGHT, P. A., D. J. RANDALL, and S. E. PERRY III. 1989. Fish gill water boundary layer: a site of linkage between carbon dioxide and ammonia excretion. J. Comp. Physiol. B 158: 627–635.

YALKOWSKY, S. H., O. S. CARPENTER, G. L. FLYNN, and T. G. SLUNICK. 1973. Drug absorption kinetics in goldfish. J. Pharm. Sci. 62: 1949–1954.

CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 47, No. 11 — article by B. D. Smith, G. A. McFarlane, and M. W. Saunders: *Variation in Pacific hake* (Merluccius productus) *summer length-at-age near southern Vancouver Island and its relationship to fishing and oceanography*.

Page 2202: Equation 7 should read as follows:

$$f = 0.5 \sum_{i,y} ((S_{i,y} - O_{i,y})/\sigma_{i,y})^2.$$

VOLUME 47, No. 12 — article by D. K. Kimura: *Approaches to age-structured sequential population analysis*. Page 2366, left column: incorrect expressions should read as follows:

Line 29: $\hat{d}_{ij} = \hat{c}_i \hat{a}_{ij}$

Line 49: $-L = -\Sigma_{ik} n_i f_{ik} \log (\hat{f}_{ik}/f_{ik}) + \Sigma_i [\log(c_i) - \log(\hat{c}_i)]^2/(2\sigma^2) + \text{const.}$

Line 52: $\hat{c}_{ik} = \hat{c}_i \hat{f}_{ik}$.

Page 2368: In the caption to Table 1, the total population numbers should read "in billions."
In the text, left column, line 18, the expression should read

$$ce\,(\hat{B}_i^t) = \text{sqrt}\,[\text{MSE}\,(\hat{B}_i^t)]/B_i^t.$$

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 48, No. 2, February 1991

Volume 48, nº 2, février 1991

Duston, J., R. L. Saunders, and D. E. Knox. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*) 164–169

García-Vázquez, E., P. Morán, and A. M. Pendás. Chromosome polymorphism patterns indicate failure of a Scottish stock of *Salmo salar* transplanted into a Spanish river 170–172

Wangberg, S.-Å., U. Heyman, and H. Blanck. Long-term and short-term arsenate toxicity to freshwater phytoplankton and periphyton in limnocorrals 173–182

Diana, J. S., D. J. Dettweiler, and C. K. Lin. Effect of Nile tilapia (*Oreochromis niloticus*) on the ecosystem of aquaculture ponds, and its significance to the trophic cascade hypothesis 183–190

Minshall, G. W., E. Hitchcock, and J. R. Barnes. Decomposition of rainbow trout (*Oncorhynchus mykiss*) carcasses in a forest stream ecosystem inhabited only by nonanadromous fish populations 191–195

Lamberti, G. A., S. V. Gregory, L. R. Ashkenas, R. C. Wildman, and K. M. S. Moore. Stream ecosystem recovery following a catastrophic debris flow 196–208

Charmantier, G., M. Charmantier-Daures, S. L. Waddy, and D. E. Aiken. Salinity tolerance and osmoregulation in the nemertean *Pseudocarcinonemertes homari*, an egg predator of American lobster, *Homarus americanus* 209–214

MacDonald, B. A., R. J. Thompson, and N. F. Bourne. Growth and reproductive energetics of three scallop species from British Columbia (*Chlamys hastata*, *Chlamys rubida*, and *Crassadoma gigantea*) 215–221

Moore, J. N., S. N. Luoma, and D. Peters. Downstream effects of mine effluent on an intermontane riparian system 222–232

Ostrofsky, M. L., and G. G. McGee. Spatial variation in the distribution of phosphorus species in the surficial sediments of Canadohta Lake, Pennsylvania: implications for internal phosphorus loading estimates 233–237

Hontela, A., J. B. Rasmussen, D. Ko, K. Lederis, and G. Chevalier. Arginine vasotocin, an osmoregulatory hormone, as a potential indicator of acid stress in fish 238–242

Johnsson, J. I., and M. V. Abrahams. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study 243–247

Solazzi, M. F., T. E. Nickelson, and S. L. Johnson. Survival, contribution, and return of hatchery coho salmon (*Oncorhynchus kisutch*) released into freshwater, estuarine, and marine environments 248–253

Dorazio, R. M., and P. J. Rago. Evaluation of a mark–recapture method for estimating mortality and migration rates of stratified populations 254–260

Hebert, C. E., and G. D. Haffner. Habitat partitioning and contaminant exposure in cyprinids 261–266

Hines, A. H. Fecundity and reproductive output in nine species of *Cancer* crabs (Crustacea, Brachyura, Cancridae) 267–275

Richard, P. R. Abundance and distribution of narwhals (*Monodon monoceros*) in northern Hudson Bay 276–283

Klump, J. V., J. L. Kaster, and M. E. Sierszen. *Mysis relicta* assimilation of hexachlorobiphenyl from sediments 284–289

Patrick, P. H., B. Sim, and G. Hunt. Range detection of targets using hydroacoustics in the laboratory 290–295

Castonguay, M., P. Simard, and P. Gagnon. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination 296–302

Anderson, J. T., and W. G. Warren. Comparison of catch rates among small and large bongo samplers for *Calanus finmarchicus* copepodite stages 303–308

Bartlett, S. E., and W. S. Davidson. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome *b* genes 309–317

Barber, M. C., L. A. Suárez, and R. R. Lassiter. Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Lake Ontario salmonids 318–337

Corrections to recent publications/Corrections sur récentes publications 338

© Minister of Supply and Services Canada 1991
Ministre des Approvisionnements et Services Canada 1991

CJFSDX48(2) 163–338 (1991)
ISSN 0706-652X

Subscription rates/Abonnements

Canada: Institutional/Collectif $210; Personal/Personnel $85
Other countries/Autres pays : Institutional/Collectif $252(U.S.); Personal/Personnel $102(U.S.)
Single issue/Par numéro : $14.95 (Canada); $17.95(U.S.) (Other countries/Autres pays)